# RISK FACTORS FOR ORAL HPV PERSISTENCE AND HPV-ASSOCIATED CANCER

by
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# Abstract

**Background:** Persistent human papillomavirus (HPV) infection is a well-known cause of cervical and anogenital cancers. In the past decade, oral HPV infection was established as a cause of oropharyngeal squamous cell carcinoma (OPSCC), but risk factors for oral HPV persistence are poorly understood. Increasing HPV-associated OPSCC has been reported in several Western countries, predominantly in younger white men. Trends in Asian populations have not been well-described, but may differ since the epidemiology of HPV-associated cancers varies by age, sex, geography and race/ethnicity.

**Objectives:** We aimed to investigate potential factors associated with oral HPV persistence, including 1) serum cytokines, 2a) recreational drug use and 2b) medication use. We also explored: 3) the epidemiology of OPSCC and other potentially HPV-associated cancers in Singapore, a Southeast Asian country.

**Methods:** For aims 1 and 2, participants were enrolled in the "Persistence of Oral Papillomavirus Study" (POPS), a U.S.-based cohort study. Oral rinse samples were collected semi-annually for HPV DNA testing. Blood samples were collected for cytokine testing. Drug and medication use were assessed via questionnaire. For aim 3, the epidemiology of potentially HPV-associated cancers in Singapore was described, using cancer registry data. Data were analyzed using Wei-Lin-Weissfeld regression (aims 1&2) and Joinpoint regression (aim 3).

**Results:** From 2010 to 2014, 1,666 participants were enrolled in POPS. Oral HPV prevalence was 36% and median time to clearance was 6.3 months. Higher TNF-α concentration was associated with decreased oral HPV clearance in men (highest vs. lowest quartile, adjusted hazard ratio [aHR]=0.52, 95%CI=0.34-0.79) and women (aHR=0.76, 95%CI=0.55-1.04), p-interaction=0.049. Cocaine use (aHR=0.60, 95%CI=0.41-0.88) and antipsychotic medication use (aHR=0.75, 95%CI=0.57-0.99) were also each associated with reduced clearance. In Singapore, OPSCC incidence increased in both genders (men 1993-2012, annual percentage change [APC]=1.9%, $p<0.001$; women 1968-2012, APC=2.0%, p=0.01), in contrast to non-oropharyngeal head and neck cancers. The incidence of HPV-associated cancers varied by ethnicity.

**Conclusions:** Some immunomodulatory agents, including high serum TNF-α, cocaine, and antipsychotic medication may reduce oral HPV clearance. Similar to trends in other countries, OPSCC incidence increased in Singapore in recent years, suggesting the epidemiologic shift in head and neck cancers is not just occurring in the West.

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# Table of Contents

# List of Tables

**Chapter 3:**



**Chapter 4:**

**Chapter 5:**



**Chapter 6:**

# List of Figures

**Chapter 4:**



**Chapter 5:**



**Chapter 6:**

# Abbreviations and acronyms

APC – annual percentage change

ART – anti-retroviral therapy

ASCC – anal squamous cell carcinoma

ANSCC – anal non-squamous cell carcinoma

HAART – highly active anti-retroviral therapy

HIV – Human Immunodeficiency Virus

HNC – head and neck cancer

HNSCC – head and neck squamous cell carcinoma

HPV – Human Papilloma Virus

ICC – invasive cervical cancer

IL – interleukin

MACS – Multicenter AIDS Cohort Study

NK cell – natural killer cell

NPC – nasopharyngeal cancer

OPSCC – oropharyngeal squamous cell carcinoma

Non-OP HNC – non-oropharyngeal head and neck carcinoma

NSAID - non-steroidal anti-inflammatory drug

PCR – polymerase chain reaction

PED – performance enhancing drug

POPS – Persistence of Oral Papillomavirus Study

Th1 – T-helper cell type 1

Th2 – T-helper cell type 2

TNF-α - Tumor necrosis factor-alpha

WIHS – Women's Interagency Health Study

WLW – Wei-Lin-Weissfeld

# Chapter 1. Introduction

Human papillomavirus (HPV) is a common sexually transmitted infection and well-known cause of cervical and other anogenital cancers.[1] In the past decade, oral infection with HPV was established as a causal factor for a subset of head and neck squamous cell carcinomas (HNSCC), particularly oropharyngeal squamous cell carcinoma (OPSCC).[2-7] In the 1990s to early 2000s, an estimated 35-41% of OPSCC were attributed to HPV,[8,9] and the proportion may now be as high as 80% in some industrialized countries.[10] Similar to HPV infection at other anatomic sites, oral HPV infection is acquired through sexual contact,[11] and the majority of infections clear in 1-2 years with no clinically significant sequelae.[10,12] However, persisting infection increases the risk for developing viral-associated malignant transformations and neoplasms.[11] Therefore, the continued presence of detectable oral HPV infection is considered a risk factor for the development of OPSCC.[13]

Our understanding of oral HPV natural history, and factors that influence oral HPV clearance or persistence, is limited. Two longitudinal studies of oral HPV infection suggest that male sex, older age, smoking, lower CD4 T cell count, and the use and duration of highly active anti-retroviral therapy (HAART) are associated with reduced oral HPV clearance.[14,15] Human immunodeficiency virus (HIV)-related immunosuppression has also been associated with reduced clearance, but it is not known whether other types of immune-modulation may have a similar effect or whether other biological and behavioral factors play a contributory role. Current evidence indicates that

the natural history of oral HPV has some similarities to that of cervical HPV, but there also appear to be some important differences.[14] Risk factor characteristics of HPV-associated HNSCC appear to differ from that of alcohol/tobacco-related HNSCC, which have been extensively documented.[3,16] Therefore, additional research is needed to examine the unique natural history of oral HPV and to describe risk factors for persistent oral HPV infection.

Epidemiologic and molecular data indicate that the proportion of OPSCC caused by HPV is increasing, predominantly among men, people of white race and younger birth cohorts.[17-20] This trend has mainly been observed in more economically developed Western countries.[17,20] From 1988 to 2004, the population-level incidence of HPV-related OPSCC in the U.S. increased by 225% (95% CI, 208% to 242%; from 0.8 per 100,000 to 2.6 per 100,000),[2] and between 2004 and 2008, nearly 3 times as many men (9,356 cases) as women (2,370 cases) were newly diagnosed each year.[21] Worldwide, ~137,000 cases of OPSCC were diagnosed in 2008,[11] with higher incidence in the U.S. and Europe as compared to some parts of Asia.[10] Although the link between HPV and OPSCC is strongly supported by molecular and epidemiologic evidence, the reasons for differences in OPSCC incidence by sex, age and geography remain unclear.

Increased knowledge on factors related to HPV persistence and population subgroups at higher risk for OPSCC may inform cancer prevention strategies.

## Research Objectives

The objectives of this dissertation research were to:

1. Investigate whether immunologic factors, as measured by cytokine expression, are associated with oral HPV clearance in a U.S.-based cohort study of HIV-infected and HIV-uninfected adults. (Chapter 3)

2.Explore whether a) recreational drug use and b) medication use are associated with oral HPV clearance in a U.S.-based cohort study of HIV-infected and HIV-uninfected adults. (Chapters 4 and 5)

3. Evaluate ethnic, age, and gender subgroups that may be at increased risk for oropharyngeal and other HPV-associated cancers in Singapore, a racially heterogeneous Asian city-state. Population-level trends in incidence are described using data from a nationally representative cancer registry. (Chapter 6)

See conceptual frameworks in Appendix A.

# Chapter 2. Overview of HPV, head and neck squamous cell carcinomas and other HPV-associated cancers

HPV is a small DNA virus that preferentially infects undifferentiated basal cells of skin and mucosal surfaces, including the lining of the anogenital tract and oral cavity.[22] Of the >150 HPV genotypes, >15 types have been associated with human cancers,[10] and overall, HPV accounts for approximately 5% (600,000 cases) of cancers worldwide annually.[23] HPV genotypes are subdivided into 2 categories: high-risk/oncogenic types and low-risk/non-oncogenic types, based on their carcinogenicity in humans.[10] Common high-risk/oncogenic types include HPV-16, 18, 31, 33, 35, 45, 52 and 58, classified mostly based on evidence of their contribution to ~91% of cervical cancers.[11] HPV16 causes the majority of HPV-related cancers.[7]

The pathobiology of oral HPV infection is thought to be similar to infection at cervical and other anatomic sites.[11] The majority of HPV infections are asymptomatic and/or benign,[24] with minimal/no viremia since infection and viral protein production are strictly confined to keratinocytes.[25] T cell responses effectively resolve the majority of infections.[26] In cases where HPV successfully evades immune clearance, E6/E7 oncoprotein expression leads to excessive viral proliferation in infected lower and middle epithelial layers,[24] which eventually spreads to surface layers and results in lesion development.[27] Deficient DNA repair and accumulation of genetic damage from these productive infections are responsible for tumor formation and cancer progression.[24]

Persistent infection with oncogenic HPV types is a necessary cause of all cervical cancers and causes the majority of anal cancers and oropharyngeal cancers.[7,13] A high proportion (~50-80%) of OPSCC is attributed to oncogenic HPV type 16,[11] possibly because immune responses clear HPV16 less efficiently than other HPV types.[12,28] Other well-studied HPV types include HPV18, which accounts for a substantial proportion (20%) of cervical cancers[29] but appears to play a small role (2.8%) in oropharyngeal cancer;[30] and, HPV6 and HPV11 which cause genital warts but do not cause cancer.[11]

Historically, the primary risk factors for HNSCC have been tobacco and alcohol use.[31] Although smoking and drinking still account for the majority of HNSCC, a decline in tobacco use in the U.S. and other countries has revealed an increasing proportion of HNSCC, primarily OPSCC, that are independently associated with HPV.[10] Consistent with this trend, OPSCC in the past decade have been more likely to be never/light/former smokers, than patients diagnosed in previous decades.[32] Compared to patients with HPV-unrelated OPSCC, patients with HPV-related OPSCC are more likely to be middle-aged white men, and have higher socioeconomic status and higher educational level.[10,23]

## Previous research on oral HPV natural history

The data available on oral HPV natural history are limited and results from cohort studies have been varied. A longitudinal study of oral HPV infections in men in the U.S., Mexico and Brazil found that the median duration of incident infections was 6.9 months (95% CI=6.2-9.3) for any HPV type, 6.3 months (6.0–9.9) for oncogenic HPV, and 7.3 months (6.0–not estimable) for HPV16.[12] A study of sexually active heterosexual men found that 72% of prevalent infections of any type cleared within ~4 months.[33] A study in

Italy found that in 30 oral HPV-infected subjects with low (3%) HPV16 prevalence, only 2 infections persisted for 6 months and complete clearance was observed within 1 year.[34] A Finnish prospective study of 331 women and 131 male spouses found that at 2 years after initial oral HPV detection, none of the women and few (5%) of the men had cleared the infection.[35] At 6 years of follow-up in this same cohort, persisting infections were primarily HPV16 (76%), and after including additional infections detected during the additional 4 years of follow-up, mean time to clearance of HPV16 infections was 18.6 months.[36] At 7 years of follow-up, 71% of the men had cleared oral HPV with most of the persisting infections caused by HPV16 (mean time to clearance: 21.7 months).[37]

## Oral HPV infection and persistence in HIV-infected individuals

Studying HPV persistence in the context of HIV infection is of particular interest since HIV-infected individuals have a 2-3 times higher odds of prevalent oral HPV infection, even after accounting for sexual risk factors, and are more likely to get OPSCC than HIV-uninfected individuals.[38,39] In the general U.S. population, ~7% of people have oral HPV infection at any one time.[40] In HIV-infected individuals, the prevalence is as high as 40%.[5,39,41] It is not clear whether the higher prevalence is due to increased acquisition or longer duration of infection.

In a study of HIV-infected men and women, 48% of prevalent infections and 28% of newly detected infections remained persistent for at least 18 months.[42] A study of women with or at risk for HIV found that current smoking, older age, lower CD4 T cell counts, and the use and duration of HAART therapy were associated with increased risk for 6-month persistence of infection.[14] Another study found that male sex, older age and

current smoking were associated with reduced clearance, while HAART use was not associated with clearance.[15] It is possible that HIV-related immunosuppression, such as lower CD4 T cell counts, may attenuate immune responses to HPV, facilitating persistence.[14] However, it is unclear whether HIV-related immunosuppression alone explains the higher prevalence of oral HPV in HIV-infected individuals, particularly since a decrease in oral HPV prevalence has not been observed in the post-HAART era.[43] It is also possible that other factors commonly associated with HIV such as drug use, smoking, alcohol use or other chronic conditions play a contributory role.

# Chapter 3. The association of serum cytokines with clearance or persistence of oral HPV infection


## Abstract

**Background:** Initial studies suggest higher serum levels of some pro-inflammatory cytokines may be associated with decreased cervical human papillomavirus (HPV) clearance. However, the relationship of cytokines with oral HPV clearance has not been explored.

**Methods:** From 2010 to 2014, oral rinse and serum samples were collected semi-annually from 1,601 adults. Oral rinse samples were tested for HPV DNA using PCR. Based on oral HPV results, 931 serum samples were selected for cytokine evaluation to include a roughly equal number of prevalent (n=307), incident (n=313), and no oral HPV infections (n=311). Electrochemiluminescence multiplex assays were used to determine the concentrations of IL-6, IL-8, TNF-α, IFN-γ, IL-1β, IL-2, IL-4, IL-10, IL-12 and IL-13. The relationship between serum cytokine concentrations (categorized into quartiles) and oral HPV clearance was evaluated with Wei-Lin-Weissfeld regression models, adjusting for HPV infection type (prevalent vs. incident), age, HIV status, and CD4 T cell count.

**Results:** Higher TNF-α concentration was associated with decreased clearance in men (highest vs. lowest quartile, adjusted hazard ratio [aHR]=0.52, 95% CI=0.34-0.79) and

women (aHR=0.76, 95% confidence interval [CI]=0.55-1.04), with stronger associations in men than women (p-interaction=0.049). Higher IL-2 concentration was associated with reduced clearance in men (aHR=0.69, 95% CI=0.50-0.95), but not women (p-interaction=0.058). Results were similar within CD4 T cell strata (CD4 ≥500 or CD4<500 cells/μl) among HIV-infected participants. No other cytokines were associated with clearance.

**Conclusion:** High serum TNF-α is associated with reduced clearance of oral HPV infection.

## Background and rationale

Oral infection with human papillomavirus (HPV) is an established causal factor for oropharyngeal squamous cell carcinoma (OPSCC).[19] In the U.S., approximately 70% of OPSCC is caused by HPV, with higher incidence among men <60 years old and individuals with human immunodeficiency virus (HIV) infection.[19,39,44,45] Similar to HPV infection at other anatomic sites, most oral HPV infections clear in 1-2 years.[1] However, some individuals have persistently detected oral HPV infection, which is believed to be the precursor to HPV-related OPSCC.[13] Understanding key immune differences in individuals with persistent oral HPV may help explain the disproportionate burden of HPV-related OPSCC in population subgroups.

While the role of host immunity in oral HPV infection has not been explored, studies of cervical HPV infection have established that cell-mediated T cell responses are critical for viral clearance and lesion regression.[26,46] Attempts to characterize these responses have found that women with persistent cervical HPV have distinct systemic and/or local cytokine profiles as compared to women who clear HPV or women without HPV infection.[47-51] In particular, higher circulating or local levels of interleukin (IL)-6, IL-8 and tumor necrosis factor-alpha (TNF-α) have been associated with reduced cervical HPV clearance,[47-49] suggesting that cytokines secreted may be predictive of disease progression and outcome, although results have been mixed.[52,53] The relationships between cytokines and oral HPV infection, and any differences by biologic sex, have not been described. However, men have been noted to have a reduced inflammatory response to infection, compared to women.[54]

Given that innate and acquired immune responses effectively resolve most HPV infections, persistent oral infection may reflect disruption or alteration of host anti-viral or inflammatory responses.[29,55] Alternatively, immunologic differences between individuals may influence oral HPV clearance. For example, women with persistent cervical HPV infection have reduced lymphoproliferative responses to HPV16.[56] HIV infection may further impact immune response, as HIV can alter cytokine secretion patterns, resulting in elevated circulating levels of pro-inflammatory cytokines such as IL-1, IL-6 and TNF-α.[43,57-62] Furthermore, pro-inflammatory cytokines can modulate HPV transcription, suggesting that HIV-related immune perturbations at the systemic level could affect oral infection.[43]

Since there is no standardized oral screening, or easily identifiable precursor lesion for OPSCC,[63,64] reliably detectable biomarkers related to infection duration may help identify oral HPV-infected individuals at increased risk for persistent infection and/or HPV-related OPSCC. Current technology allows simultaneous measurement of multiple immune markers in a single sample using a multiplex format. Characterizing the serum cytokine profiles associated with oral HPV clearance may provide insight on immune cofactors relevant to oral HPV infection and identify important biomarkers for future research. The goals of this study were to evaluate whether select serum cytokines associated with inflammation may be related to oral HPV clearance and to explore differences by sex and degree of HIV-related immunosuppression as measured by CD4 T cell count.

## Methods

*Study population*

From 2010 to 2014, 1,601 participants were enrolled in the "Persistence of Oral Papillomavirus Study" (POPS), a longitudinal cohort for studying oral HPV natural history. POPS was nested within two ongoing observational studies of men and women with or at risk for HIV: the Multicenter AIDS Cohort Study (MACS) and the Women's Interagency HIV Study (WIHS). POPS participants were enrolled at 5 study centers: Baltimore (MACS), Pittsburgh (MACS), Brooklyn (WIHS), Bronx (WIHS), and Chicago (MACS and WIHS).[65-67] Each study center's Institutional Review Board approved the study. All participants provided written informed consent.

*Data collection*

At each semi-annual study visit, a 30-second Scope® oral rinse and gargle sample was collected and tested for 37 types of HPV DNA. A blood sample was also obtained, from which HIV status, CD4 T cell count, and cytokine profile were determined. Data on behavioral cofactors were collected by survey. Surveys were administered via Computer-assisted Self-interviewing (CASI) in the MACS and were interview-administered in the WIHS.

*Oral HPV detection and genotyping*

DNA was extracted from oral exfoliated cells using the Puregene DNA isolation kit (Gentra Systems, Minneapolis, MN). Extracted DNA samples were tested for HPV DNA using a polymerase chain reaction (PCR) assay targeting the L1 region of the viral

genome using PGMY primers.[68] HPV-positive samples were genotyped using reverse line-blot hybridization (Roche Molecular Systems, Pleasanton, CA).[68] Each sample was evaluated for the presence of high-risk/oncogenic types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66) and low-risk/non-oncogenic types (6, 11, 26, 38, 40, 42, 53, 54, 55, 61, 62, 64, 67, 68, 69, 70, 71, 72, 73, 81, 83, 84, 89, IS39), classified according to criteria from the International Agency for Research on Cancer.[7,69,70] Participants with any of these HPV types were considered HPV-infected.

Prevalent oral infections were defined as any type-specific oral HPV infection detected at study enrollment. For the purposes of this study, incident infections were defined as those preceded by at least one HPV-negative oral rinse, although it is possible some of these were re-expressed latent infections. Previous studies of oral HPV natural history have reported intermittent detection of oral HPV (i.e. HPV positive/negative/positive), although it is unclear whether this is due to reactivation of latent infection, newly-acquired infection, fluctuations in HPV viral load over time or assay limitations.[34,36,42] In order to reduce misclassification bias, re-infection(s) with the same HPV type after clearance was not included in the analysis.

Clearance was defined as two consecutive HPV-negative oral rinses, with time of clearance as the visit of the first negative oral rinse sample. A secondary less conservative definition of clearance was considered, requiring only one HPV-negative oral rinse sample. The duration of infection was defined as elapsed time from oral HPV DNA detection to HPV clearance, study end or the last follow-up time point.

*Selection of serum samples for cytokine testing*

We selected 931 serum samples for cytokine evaluation. Serum samples were selected based on oral rinse HPV results in order to include a roughly equal number of prevalent (n=307), incident (n=313), and no oral HPV infections (n=311). We included all prevalent HPV samples, a random selection of incident HPV samples (55% of all incident HPV samples), and a comparison group of samples with no oral HPV DNA at study enrollment, frequency matched to samples with prevalent HPV by study participant's HIV status and biologic sex. All of the serum samples selected for cytokine testing were from participants who contributed at least 3 oral rinses overall and at least 2 oral rinses after incident oral HPV detection.

Cytokines were evaluated using serum samples collected at the visit of incident HPV detection, or at study enrollment for participants with prevalent or no HPV infections. Some participants had prevalent infection at study enrollment and subsequent incident HPV that was also included in the study sample. For these cases, cytokines were determined separately in the serum sample corresponding to first detection of prevalent HPV and in the sample corresponding to incident HPV. Therefore, the 620 prevalent and incident serum samples selected for cytokine evaluation were obtained from 459 HPV-infected participants.

*Serum cytokine testing*

Serum cytokines were determined using the MSD V-PLEX Human Proinflammatory Panel 1 kit (Meso Scale Discovery -MSD Gaithersburg, MD), which

consists of the following: TNF-α, IL-6, IL-8, IFN-γ, IL-1β, IL-2, IL-4, IL-10, IL-12 and IL-13. Cytokine testing was analyzed using the MSD 2400 Sector Imager using a standard protocol. The multiplex cytokine assay was previously validated and found to reliably quantify cytokine concentrations in both HIV-infected and HIV-uninfected individuals.[71]

To confirm acceptable assay performance, 10% of the samples were tested in duplicate and coefficients of variation (CV) were calculated for each duplicate test. Duplicated cytokine results were considered acceptable if CV≤20%. Analysis was restricted to the cytokines that could be reliably detected in ≥60% of the samples tested: TNF-α, IL-6, IL-8, IFN-γ, IL-2 and IL-10. The other cytokines tested (IL-1β, IL-4, IL-12 and IL-13) were excluded from the analysis because >40% of samples had cytokine concentrations below the lowest detectable limit (Table 3-2).

*Statistical analyses*

Demographic and health characteristics of men and women in the study were compared using $\chi^2$ tests. Median cytokine concentrations were compared in serum samples that had prevalent, incident and no oral HPV DNA using a nonparametric test for trends across ordered groups.

The analysis included 917 type-specific oral HPV infections from 620 samples among 459 participants (some participants were infected with multiple HPV types at the same visit and the cytokine values at that visit were applied to clearance analysis of all of those infections). Concentrations of each cytokine were categorized into quartiles (see Table 3-6 footnote for quartile cutoffs). Alternative analyses considered cytokine

quartiles based on sex-specific cytokine distributions and continuous cytokine concentrations (i.e. the per natural log increase in cytokine concentration), with similar results.

Predictors of type-specific oral HPV clearance were explored using Wei-Lin-Weissfeld (WLW) regression models, accounting for within-participant clustering of HPV infections.[72] The effect of each cytokine was modeled separately in unadjusted models, and then in multivariable models adjusting for prevalent vs. incident infection type, age, HIV status, and CD4 T cell count at time of oral HPV detection, based on prior evidence that these factors are associated with oral HPV clearance. Stata 12 statistical software package was used for statistical analyses.[73] Statistical significance was defined by a two-sided p-value $<0.05$.

## Results

Characteristics of study participants are described in Table 3-1. Among the 766 participants, half were men (52%) and the median age was 50 years. Approximately 74% of participants were HIV-infected, and 82% of these reported current use of antiretroviral therapy (ART). Compared to men, women were younger, more likely to be Black, HIV-infected, and current smokers (p-values$<0.05$, Table 3-1). Among HIV-infected participants, women had higher current HIV RNA viral load ($p<0.0001$), but similar CD4 T cell count, as compared to men ($p=0.17$).

Median follow-up time for infections was 16.7 months (interquartile range, IQR: 6.1-36.6). For infections that cleared, the median time to clearance was 6.5 months, with

longer time to clearance for prevalent infections (11.7 months; IQR 6.0-21.6) than incident infections (6.2 months; IQR 5.9-11.9).

*Comparison of serum cytokine concentrations by HPV status*

Median serum concentrations of TNF-α, IL-8, IFN-γ, IL-10 and IL-2 increased by HPV infection status (each p-trend<0.05, Table 3-3). The lowest median concentrations of each of these cytokines were in samples from participants with no oral HPV infection, followed by samples from participants with incident HPV, and the highest concentrations were in samples from participants with prevalent HPV. When comparing samples with any oral HPV infection (prevalent or incident) to samples without oral HPV infection, the median serum concentrations of TNF-α, IL-8, IFN-γ, IL-10 and IL-2 were significantly higher (each p<0.05) in samples from HPV-infected participants (Table 3-4).

*Association of cytokines with oral HPV clearance*

Of the cytokines included in the analysis, TNF-α was most strongly associated with oral HPV clearance (Table 3-5). In unadjusted analyses, the highest quartile of TNF-α was significantly associated with reduced oral HPV clearance in both men (HR=0.50, 95% CI=0.35-0.72) and women (HR=0.67, 95% CI=0.48-0.94), as compared to the lowest TNF-α quartile (p-interaction=0.24). The second and third quartiles of TNF-α concentration were also associated with reduced oral HPV clearance in men but not in women (each p-interaction<0.05, Table 3-5).

In unadjusted analyses, the highest quartile of IL-2 concentration was associated with significantly reduced oral HPV clearance among men (HR=0.69, 95% CI=0.50-

0.96), but not among women (HR=1.10, 95% CI=0.80-1.50), p-interaction=0.047. This finding should be interpreted with caution as IL-2 had the most values (35%) below the lower limit of detection, of all the cytokines included in the analysis. IL-6, IL-8, IFN-γ, and IL-10 concentration were not strongly associated with oral HPV clearance; marginal associations were observed in single quartiles for some of these cytokines in unadjusted analyses (Table 3-5), but none remained significant predictors of oral HPV clearance in adjusted analyses (results not shown).

After adjusting for the effects of HPV infection type (prevalent vs. incident), age, HIV status and CD4 T cell count in a multivariable model, serum TNF-α concentration remained significantly associated with reduced oral HPV clearance in all quartiles for men (each $p<0.01$) and remained marginally associated with reduced clearance in the highest quartile for women (p-interaction=0.049, Table 3-6). IL-2 also remained associated with decreased oral HPV clearance in all quartiles in men only (each $p<0.05$). The associations of higher TNF-α and IL-2 concentrations on oral HPV clearance were stronger in men than women (TNF-α, p-interaction=0.049; IL-2, p-interaction=0.058).

Results were similar when restricted to HIV-infected participants with baseline CD4 ≥500 or CD4<500 cells/μl (results not shown) and when evaluating TNF-α and IL-2 concentrations as continuous variables instead of quartiles (Table 3-7). When restricted to oncogenic HPV types, higher TNF-α remained associated with reduced clearance in men, but not in women although interaction by oncogenic type was not statistically significant (p-interactions, men=0.96; women=0.29). When using sex-specific cytokine quartile cutoffs, results were also similar but the highest TNF-α quartile in women remained significantly associated with reduced clearance in the adjusted model (p=0.02, Table 3-8).

When using a less strict clearance definition of 1 HPV-negative oral rinse, similar patterns were observed, but associations were attenuated (data not shown).

## Discussion

We found that while most cytokines were not associated with oral HPV clearance, the highest serum TNF-α concentration quartile in our study population (4.94-19.33 pg/ml) was associated with decreased oral HPV clearance in both sexes, with stronger associations in men than women. IL-2 concentration above the first quartile (≥0.21 pg/ml) was associated with reduced oral HPV clearance in men only. These results suggest that higher levels of some inflammatory cytokines may decrease oral HPV clearance.

Our finding that higher TNF-α was associated with reduced oral HPV clearance is consistent with previous studies of cervical HPV, which found that women with persistent cervical HPV have higher serum TNF-α at their final study visit, as compared to women who cleared infection.[47,48] In addition, our results show that higher serum TNF-α at first detection of oral HPV infection was associated with longer time to clearance, suggesting that TNF-α may be a relevant marker in early stages of oral HPV infection, in addition to being a marker of long-term infection. Furthermore, while previous studies restricted the study population to HIV-uninfected participants,[47-49,52] the association between TNF-α and clearance in our study remained similar after adjusting for HIV infection and CD4 T cell count, suggesting that the relationship between TNF-α and oral HPV may be independent of HIV-related immunosuppression.

TNF-α plays an important role in inflammatory reactions and viral clearance. Since HPV infection of epithelial cells is sequestered from systemic immunity, and typically does not cause a highly inflammatory immune response,[74,75] the reason why higher concentrations of TNF-α are associated with reduced clearance remains unclear. It is possible that these participants had higher systemic TNF-α resulting from other pro-inflammatory co-factors, such as concurrent illness or chronic conditions, which may promote or facilitate HPV persistence. Previous research suggests that although initial HPV infection does not cause an inflammatory reaction,[74,75] persisting infection can activate inflammatory pathways, including ones with established roles in cancer promotion.[76] Repeated longitudinal cytokine measurements, or cytokine measurements taken directly from the oropharynx, may show a decrease in TNF-α following HPV clearance, and may help clarify temporal patterns of immune response over the course of oral HPV infection.

Some sex differences in the association of cytokines with oral HPV clearance were observed in this study. Genetic or hormonal differences that influence immune responses and HPV infection may explain the difference between men and women in strength of association of TNF-α and IL-2 with reduced clearance. Sex differences in cellular and humoral immune responses, as well as cytokine activity, have been consistently documented in previous research.[54,77,78] Given that our study population was primarily middle-aged, it is also possible that hormonal changes related to oral contraceptive use and menopause in women might influence immune responses.[79,80]

Previous clinical studies have not found evidence that serum IL-2 may be related to clearance of HPV infection. However, a previous study that measured IL-2 reactivity

when stimulated with HPV E6/E7 oncoproteins in culture supernatants of peripheral blood mononuclear cells found that IL-2 responsiveness was strongly associated with cervical HPV16 persistence.[81] Other studies have shown that plasma IL-2 levels can differ depending on cervical HPV positivity and/or disease stage, although results have varied.[51] Our finding that IL-2 is related to decreased clearance in men may have been affected by its lower detectability, and may not represent true sex differences in IL-2. Therefore, the association of IL-2 with oral HPV clearance observed in this study warrants confirmation from other studies.

In contrast to previous studies on cervical HPV,[47-49] IL-6 and IL-8 were not strongly associated with oral HPV clearance in this study. It is possible that these cytokines were expressed at time points not captured by the testing schedules, that concentrations differed between studies or that they may be involved in oral HPV clearance, but not at concentrations detectable in serum. Two previous analyses, which reported associations between IL-8 and reduced cervical HPV clearance were conducted in a weighted subpopulation of women with type-specific HPV infection persistently detected over a 9-year interval.[47,48] Therefore, it is possible that IL-8 is associated with HPV infection at a later stage, or may be more relevant for established infections. We found that the concentrations of nearly all cytokines were indeed different across participants by HPV infection status, suggesting a linkage between cytokine profile and presence or duration of HPV infection. While the current analysis controlled for incident vs. prevalent infection type, longitudinal repeated measurements may be more informative of differences between short- and long-term persistence.

Our study had several strengths, including: enrollment of a large study population with high oral HPV prevalence, including men and women at multiple U.S. study sites; racial and ethnic diversity; inclusion of HIV-infected and HIV-uninfected participants; ability to control for biological and behavioral risk factors; evaluation of oral HPV infection in a prospective manner; and, centralized testing of cytokines using a validated multiplex assay. This study also had several limitations. Cytokine levels were only measured in serum, not saliva, although results of a recent study indicate that serum cytokine measurement may reflect markers detectable in oral samples.[82] It remains uncertain how cytokine concentrations in the periphery may be related to viral clearance at the local site of infection, or how clearance may be affected by the contributing effects of the mucosal immunologic environment. Cytokines were measured at the time of first oral HPV detection so subsequent changes in cytokine concentration during infection could not be evaluated. Finally, the 6-month study visit intervals limited our ability to identify time of infection and time of clearance with greater precision.

## Conclusion

To our knowledge, this is the first study to explore the association of circulating immune markers with oral HPV clearance. While the majority of cytokines were not related to oral HPV clearance, these results suggest higher TNF-α concentration may be associated with reduced clearance of oral HPV infection, and that the strength of association may be stronger in men than women. Future studies, which include

longitudinal cytokine evaluation, are needed to clarify the role of TNF-α in oral HPV natural history.


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(WIHS). WIHS (Principal Investigators): U01-AI-103408; Bronx WIHS (Kathryn Anastos), U01-AI-035004; Brooklyn WIHS (Howard Minkoff and Deborah Gustafson), U01-AI-031834; Chicago WIHS (Mardge Cohen and Audrey French), U01-AI-034989; WIHS Data Management and Analysis Center (Stephen Gange and Elizabeth Golub), U01-AI-042590. The WIHS is funded primarily by the National Institute of Allergy and Infectious Diseases (NIAID), with additional co-funding from the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD), the National Cancer Institute (NCI), the National Institute on Drug Abuse (NIDA), and the National Institute on Mental Health (NIMH).

**Table 3-1.** Description of study participant characteristics at baseline, overall and by sex[1]

| Characteristic | n (%) | | | |
|---|---|---|---|---|
| | **Overall (N=766)** | **Men (N=402)** | **Women (N=364)** | **p-value** |
| **Infection type** | | | | 0.88 |
| Incident only | 152 (19.8) | 77 (19.2) | 75 (20.6) | |
| Any prevalent | 307 (40.1) | 163 (40.5) | 144 (39.6) | |
| None | 307 (40.1) | 162 (40.3) | 145 (39.8) | |
| **Age (years)** | | | | **<0.0001** |
| <45 | 200 (26.1) | 58 (14.5) | 142 (39.0) | |
| 45-54 | 328 (42.9) | 171 (42.6) | 157 (43.1) | |
| ≥55 | 238 (31.1) | 173 (43.0) | 65 (17.9) | |
| **Race/Ethnicity** | | | | **<0.0001** |
| White, non-Hispanic | 254 (33.2) | 238 (59.2) | 16 (4.4) | |
| Black, non-Hispanic | 413 (53.9) | 144 (35.8) | 269 (73.9) | |
| Other race or Hispanic | 99 (12.9) | 20 (5.0) | 79 (21.7) | |
| **Study site** | | | | |
| Baltimore, Maryland | 140 (18.3) | 140 (34.8) | NA | |
| Bronx, New York | 116 (15.1) | NA | 116 (31.9) | |
| Brooklyn, New York | 134 (17.5) | NA | 134 (36.8) | |
| Chicago, Illinois | 253 (33.0) | 139 (34.6) | 114 (31.3) | |
| Pittsburgh, Pennsylvania | 123 (16.1) | 123 (30.6) | NA | |
| **HIV status** | | | | **0.02** |
| Uninfected | 197 (25.7) | 117 (29.1) | 80 (22.0) | |
| Infected | 569 (74.3) | 285 (70.9) | 284 (78.0) | |
| **Current CD4 T cell count (cells/µL)** | | | | 0.17 |
| ≥500 | 313 (55.0) | 165 (57.9) | 148 (52.1) | |
| <500 | 256 (45.0) | 120 (42.1) | 136 (47.9) | |
| **Current HIV RNA viral load (copies/mL)** | | | | **<0.0001** |
| <200 | 415 (73.3) | 228 (80.9) | 187 (66.5) | |
| 200-19,999 | 94 (16.7) | 32 (11.3) | 62 (22.1) | |
| ≥20,000 | 54 (9.6) | 22 (7.8) | 32 (11.4) | |
| **Current ART use** | | | | 0.43 |
| No | 101 (17.8) | 47 (16.5) | 54 (19.0) | |
| Yes | 468 (82.2) | 238 (83.5) | 230 (81.0) | |
| **Smoked cigarettes in the last 6 months** | | | | **<0.0001** |
| No | 439 (57.8) | 271 (68.3) | 168 (46.3) | |
| Yes | 321 (42.2) | 126 (31.7) | 195 (53.7) | |

[1] For HPV-uninfected participants and participants with prevalent HPV, baseline was defined as POPS enrollment. For participants with incident HPV, baseline was defined as visit of incident HPV detection.

**Table 3-2.** Detectability of cytokines in 931 samples measured using electrochemiluminescence multiplex assays

| Cytokine | Percent (%) Detectable[1] |
|---|---|
| **TNF-α** | **99.9%** |
| **IL-6** | **98.0%** |
| **IL-8** | **97.8%** |
| **IFN-γ** | **92.9%** |
| **IL-10** | **84.5%** |
| **IL-2** | **64.4%** |
| IL-13 | 31.3% |
| IL-1β | 27.9% |
| IL-12 | 21.8% |
| IL-4 | 15.0% |

[1] The lower limit of detection was defined as the lowest reliably detected standard. The upper limit of detection was specified by the manufacturer (Meso Scale Discovery -MSD Gaithersburg, MD). The detectability of the main cytokines of interest was not affected by HIV status, sex, incident/prevalent infection type, or age.

**Table 3-3.** Comparison of baseline serum cytokine concentrations (pg/ml) in 931 samples, by oral HPV status[1]

| | **Median serum concentration at baseline (IQR)** | | | |
|---|---|---|---|---|
| **Cytokine** | **No Oral HPV n=311** | **Incident Oral HPV n=313** | **Prevalent Oral HPV n=307** | **p-trend** |
| TNF-α | **3.1 (2.4-4.3)** | **3.3 (2.5-4.5)** | **3.5 (2.6-4.8)** | **0.009** |
| IL-6 | 1.0 (0.7-1.8) | 1.1 (0.7-1.7) | 1.1 (0.7-1.7) | 0.41 |
| IL-8 | **15.2 (10.7-38.1)** | **16.4 (11.4-37.5)** | **19.5 (13.0-62.3)** | **0.001** |
| IFN-γ | **8.0 (5.2-14.1)** | **8.2 (5.4-15.7)** | **9.4 (5.7-16.5)** | **0.03** |
| IL-10 | **0.47 (0.27-0.76)** | **0.55 (0.32-0.89)** | **0.60 (0.37-0.94)** | **0.001** |
| IL-2 | **0.27 (0-0.55)** | **0.34 (0-0.55)** | **0.38 (0-0.59)** | **0.007** |

[1] For samples with no oral HPV or prevalent HPV, baseline was defined as POPS enrollment. For samples with incident HPV, baseline was defined as visit of incident HPV detection. Unadjusted (no log transformation) median cytokine concentrations and interquartile ranges (IQR, 25th to 75th percentiles, in parentheses) are shown. Bolding indicates values with $p<0.05$ in tests for trend. Results were similar when restricted to only HIV-infected, or only HIV-uninfected participants (results not shown).

**Table 3-4.** Comparison of baseline serum cytokine concentrations (pg/ml) in 931 samples, oral HPV-uninfected vs. oral HPV-infected[1]

| | **Median serum concentration at baseline (IQR)** | | |
|---|---|---|---|
| **Cytokine** | **No Oral HPV n = 311** | **Oral HPV-infected n=620** | **p-value** |
| TNF-α | **3.1 (2.4-4.3)** | **3.4 (2.5-4.7)** | **0.02** |
| IL-6 | 1.0 (0.7-1.8) | 1.1 (0.7-1.7) | 0.6 |
| IL-8 | **15.2 (10.7-38.1)** | **17.6 (12.0-46.7)** | **0.02** |
| IFN-γ | **8.0 (5.2-14.1)** | **8.9 (5.5-16.1)** | **0.03** |
| IL-10 | **0.5 (0.3-0.8)** | **0.6 (0.3-0.9)** | **0.002** |
| IL-2 | **0.3 (0.0-0.5)** | **0.4 (0.0-0.6)** | **0.04** |

[1] For samples with no oral HPV, baseline was defined as POPS enrollment. For samples with HPV, baseline was defined as visit of HPV detection. Unadjusted (no log transformation) median cytokine concentrations and interquartile ranges (IQR, 25th to 75th percentiles, in parentheses) are shown. Bolding indicates values with $p<0.05$ in tests for trend. Results were similar when restricted to only HIV-infected, or only HIV-uninfected participants (results not shown).

**Table 3-5.** Unadjusted association of cytokine concentration (by quartile) with oral HPV clearance among 459 participants with 917 oral HPV infections, by sex[1]

| | Hazard ratio (95% CI) | | | | | |
|---|---|---|---|---|---|---|
| | Infections in Men, n=472 | | | Infections in Women, n=445 | | |
| **Cytokine** | **Quartile 2** | **Quartile 3** | **Quartile 4** | **Quartile 2** | **Quartile 3** | **Quartile 4** |
| TNF-α | **0.55 (0.40-0.76)** | **0.56 (0.40-0.78)** | **0.50 (0.35-0.72)** | 0.93 (0.67-1.30) | 0.97 (0.68-1.39) | **0.67 (0.48-0.94)** |
| IL-6 | **0.72 (0.53-0.99)** | 0.71 (0.49-1.02) | 0.83 (0.61-1.12) | 1.41 (0.97-2.05) | 1.04 (0.71-1.52) | 1.05 (0.71-1.54) |
| IL-8 | 0.88 (0.64-1.20) | 0.74 (0.51-1.07) | 1.05 (0.75-1.48) | 0.83 (0.58-1.20) | 0.95 (0.71-1.29) | **0.73 (0.53-1.00)** |
| IFN-γ | 0.97 (0.70-1.34) | 0.98 (0.69-1.40) | 0.79 (0.53-1.17) | 0.79 (0.54-1.14) | 0.86 (0.63-1.17) | 0.84 (0.61-1.16) |
| IL-10 | 0.78 (0.58-1.06) | 0.81 (0.58-1.14) | 0.82 (0.58-1.15) | 0.89 (0.63-1.24) | 0.88 (0.61-1.25) | **0.74 (0.55-1.00)** |
| IL-2 | **0.63 (0.42-0.93)** | **0.73 (0.54-1.00)** | **0.69 (0.50-0.96)** | 0.80 (0.52-1.22) | 0.98 (0.71-1.36) | 1.10 (0.80-1.50) |

[1] Reference is quartile 1. Bolding indicates hazard ratios with $p<0.05$.

**Table 3-6.** Multivariable risk factors for oral HPV clearance among 459 participants with 917 oral HPV infections, by sex[1]

| Characteristic at visit of first oral HPV detection | Infections in Men, n=472 | | | Infections in Women, n=445 | | |
|---|---|---|---|---|---|---|
| | No. of cleared infections | Person-months | aHR (95% CI) | No. of cleared infections | Person-months | aHR (95% CI) |
| **TNF-α concentration (pg/ml)[2]** | | | | | | |
| Quartile 1 | 115 | 2,557 | Ref | 59 | 1,443 | Ref |
| Quartile 2 | 77 | 3,593 | **0.56 (0.40-0.79)** | 69 | 1,869 | 0.97 (0.70-1.34) |
| Quartile 3 | 62 | 2,713 | **0.60 (0.44-0.82)** | 84 | 2,392 | 1.07 (0.76-1.49) |
| Quartile 4 | 46 | 2,432 | **0.52 (0.34-0.79)** | 81 | 3,264 | 0.76 (0.55-1.04) |
| | p-interaction=0.049 | | | | | |
| **IL-2 concentration (pg/ml)[3]** | | | | | | |
| Quartile 1 | 124 | 3,612 | Ref | 101 | 3,053 | Ref |
| Quartile 2 | 39 | 1,868 | **0.66 (0.45-0.96)** | 33 | 1,243 | 0.90 (0.58-1.37) |
| Quartile 3 | 69 | 2,703 | **0.70 (0.52-0.93)** | 79 | 2,442 | 1.09 (0.82-1.47) |
| Quartile 4 | 65 | 2,906 | **0.69 (0.50-0.95)** | 79 | 2,223 | 1.15 (0.85-1.57) |
| | p-interaction=0.058 | | | | | |
| **Infection type** | | | | | | |
| Incident | 140 | 2,908 | Ref | 148 | 2,480 | Ref |
| Prevalent | 160 | 8,388 | **0.52 (0.40-0.67)** | 146 | 6,494 | **0.45 (0.34-0.59)** |
| **Age (years)** | | | | | | |
| <45 | 63 | 1,995 | Ref | 107 | 2,929 | Ref |
| 45-54 | 124 | 4,423 | 0.83 (0.61-1.12) | 137 | 4,036 | 0.95 (0.74-1.22) |
| ≥55 | 113 | 4,877 | **0.67 (0.48-0.92)** | 50 | 2,009 | 0.82 (0.58-1.15) |
| **HIV and current CD4 cell count (cells/μL)** | | | | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| HIV uninfected | 74 | 2,326 | Ref | 53 | 1,370 | Ref |
| HIV infected, CD4≥500 | 124 | 4,386 | 0.89 (0.66-1.21) | 93 | 3,157 | 0.78 (0.56-1.08) |
| HIV infected, CD4<500 | 102 | 4,584 | 0.87 (0.64-1.19) | 148 | 4,447 | 0.92 (0.68-1.25) |

[1] TNF-α and IL-2 were each analyzed in separate multivariable models adjusting for infection type, age, and HIV status/CD4 count. Covariates shown are from the model with TNF-α. Bolding indicates adjusted hazard ratios (aHR) with $p<0.05$.
[2] TNF-α quartiles: 0.0-2.56 (Q1), 2.57-3.58 (Q2), 3.59-4.93 (Q3), 4.94-19.33 (Q4).
[3] IL-2 quartiles: 0.0-0.0 (Q1), 0.21-0.37 (Q2), 0.38-0.58 (Q3), 0.59-20.62 (Q4).

**Table 3-7.** Multivariable risk factors for oral HPV clearance with continuous cytokine values, by sex

| Characteristic at visit of first oral HPV detection | Men, n=472 | | | Women, n=445 | | |
|---|---|---|---|---|---|---|
| | No. of cleared infections | Person-months | aHR (95% CI) | No. of cleared infections | Person-months | aHR (95% CI) |
| **Ln TNF-α concentration (pg/ml)[1]** | 300 | 11,272 | **0.58 (0.40-0.85)** | 293 | 8,969 | 0.87 (0.72-1.05) |
| | | | p-interaction=0.045 | | | |
| **Ln IL-2 concentration (pg/ml)[1]** | 297 | 11,066 | **0.86 (0.75-0.98)** | 292 | 8,963 | 1.01 (0.90-1.13) |
| | | | p-interaction=0.067 | | | |
| **Infection type** | | | | | | |
| Incident | 140 | 2,908 | Ref | 148 | 2,480 | Ref |
| Prevalent | 160 | 8,388 | **0.51 (0.39-0.66)** | 146 | 6,494 | **0.45 (0.34-0.59)** |
| **Age (years)** | | | | | | |
| <45 | 63 | 1,995 | Ref | 107 | 2,929 | Ref |
| 45-54 | 124 | 4,423 | 0.83 (0.62-1.12) | 137 | 4,036 | 0.95 (0.74-1.21) |
| ≥55 | 113 | 4,877 | **0.70 (0.51-0.95)** | 50 | 2,009 | 0.80 (0.56-1.13) |
| **HIV and current CD4 cell count (cells/μL)** | | | | | | |
| HIV uninfected | 74 | 2,326 | Ref | 53 | 1,370 | Ref |
| HIV infected, CD4≥500 | 124 | 4,386 | 0.90 (0.67-1.21) | 93 | 3,157 | 0.79 (0.57-1.11) |
| HIV infected, CD4<500 | 102 | 4,584 | 0.85 (0.62-1.15) | 148 | 4,447 | 0.91 (0.66-1.24) |

[1] TNF-α and IL-2 concentrations were natural log transformed. For log transformation, values of 0 were considered to be half the lower limit of detection. TNF-α and IL-2 were each analyzed in separate multivariable models adjusting for infection type, age, and HIV status/CD4 count. Covariates shown are from the model with TNF-α. Bolding indicates adjusted hazard ratios (aHR) with $p<0.05$.

**Table 3-8.** Multivariable risk factors for oral HPV clearance with sex-specific cytokine quartiles, by sex[1]

| Characteristic at visit of first oral HPV detection | Infections in Men, n=472 | | | Infections in Women, n=445 | | |
|---|---|---|---|---|---|---|
| | No. of cleared infections | Person-months | aHR (95% CI) | No. of cleared infections | Person-months | aHR (95% CI) |
| **TNF-α concentration (pg/ml)[2]** | | | | | | |
| Quartile 1 | 115 | 2,557 | Ref | 59 | 1,443 | Ref |
| Quartile 2 | 77 | 3,593 | **0.55 (0.39-0.78)** | 69 | 1,869 | 0.86 (0.63-1.17) |
| Quartile 3 | 62 | 2,713 | **0.59 (0.42-0.82)** | 84 | 2,392 | 0.95 (0.66-1.35) |
| Quartile 4 | 46 | 2,432 | **0.51 (0.35-0.73)** | 81 | 3,264 | **0.69 (0.50-0.94)** |
| | | | p-interaction=0.15 | | | |
| **IL-2 concentration (pg/ml)[3]** | | | | | | |
| Quartile 1 | 124 | 3,612 | Ref | 101 | 3,053 | Ref |
| Quartile 2 | 39 | 1,868 | **0.64 (0.42-0.96)** | 33 | 1,243 | 1.04 (0.72-1.50) |
| Quartile 3 | 69 | 2,703 | **0.69 (0.52-0.93)** | 79 | 2,442 | 1.08 (0.81-1.43) |
| Quartile 4 | 65 | 2,906 | **0.71 (0.52-0.96)** | 79 | 2,223 | 1.09 (0.80-1.50) |
| | | | p-interaction=0.07 | | | |
| **Infection type** | | | | | | |
| Incident | 140 | 2,908 | Ref | 148 | 2,480 | Ref |
| Prevalent | 160 | 8,388 | **0.51 (0.39-0.66)** | 146 | 6,494 | **0.45 (0.34-0.59)** |
| **Age (years)** | | | | | | |
| <45 | 63 | 1,995 | Ref | 107 | 2,929 | Ref |
| 45-54 | 124 | 4,423 | 0.79 (0.58-1.07) | 137 | 4,036 | 0.97 (0.75-1.24) |
| ≥55 | 113 | 4,877 | **0.67 (0.49-0.92)** | 50 | 2,009 | 0.82 (0.58-1.15) |
| **HIV and current CD4 cell count (cells/μL)** | | | | | | |
| HIV uninfected | 74 | 2,326 | Ref | 53 | 1,370 | Ref |

| | | | | | | |
|---|---|---|---|---|---|---|
| HIV infected, CD4≥500 | 124 | 4,386 | 0.91 (0.67-1.23) | 93 | 3,157 | 0.80 (0.57-1.12) |
| HIV infected, CD4<500 | 102 | 4,584 | 0.86 (0.63-1.18) | 148 | 4,447 | 0.92 (0.67-1.26) |

[1] TNF-α and IL-2 were each analyzed in separate multivariable models adjusting for infection type, age, and HIV status/CD4 count. Covariates shown are from the model with TNF-α. Bolding indicates adjusted hazard ratios (aHR) with $p<0.05$.

[2] TNF-α quartiles in men: 0.0-2.49 (Q1), 2.50-3.19 (Q2), 3.20-4.39 (Q3), 4.40-14.58 (Q4). TNF-α quartiles in women: 0.0-2.83 (Q1), 2.84-3.94 (Q2), 3.95-5.62 (Q3), 5.63-19.33 (Q4).

[3] IL-2 quartiles in men: 0.0-0.0 (Q1), 0.21-0.36 (Q2), 0.37-0.56 (Q3), 0.57-20.62 (Q4). IL-2 quartiles in women: 0.0-0.0 (Q1), 0.21-0.42 (Q2), 0.43-0.60 (Q3), 0.61-5.02 (Q4).

# Chapter 4. The association of recreational drug use with clearance or persistence of oral HPV infection


## Abstract

**Background:** Persistent oral human papillomavirus (HPV) infection increases risk for oropharyngeal squamous cell carcinoma (OPSCC). Modifiable risk factors for oral HPV persistence are not well understood, but may play an important role in HPV-related OPSCC prevention. Recreational drug use can have immunomodulatory effects, but its effect on oral HPV infection has not been previously explored. Since recreational drug use and HPV-related OPSCC prevalence are higher in HIV-infected individuals, we sought to determine whether drug use influences the natural history of oral HPV among individuals with or at risk for HIV.

**Methods:** From 2010 to 2014, Scope® oral rinse and gargle samples were collected from 1,666 participants semi-annually. Samples were tested for 37 types of oral HPV DNA using PCR with PGMY primers followed by reverse line blot hybridization. At each visit, data were collected on recent (past 6 months) drug use (alcohol, cigarette, marijuana, crack, cocaine, heroin, amphetamines, ecstasy/club drugs and speedball; in men only, poppers and sexual performance-enhancing drugs). The relationship between drug use and oral HPV clearance was evaluated with Wei-Lin-Weissfeld regression models, accounting for within-participant clustering of HPV infections. The final multivariable model adjusted for biologic sex, HPV infection type (prevalent vs.

incident), age (<45, 45-54, ≥55), HIV status, CD4 T cell count (≥500, <500 cells/μL), and oral health (saliva amount: normal/too little/too much).

**Results:** A total of 1,358 type specific oral HPV infections in 594 participants were detected and followed for 18,465 person-months. At time of first oral HPV detection, participants with HPV DNA detected reported recent use of alcohol (65%), cigarettes (47%), marijuana (27%), crack (10%), cocaine (6%), heroin (2%), amphetamines (2%), ecstasy/club drugs (0.005%) and speedball (0.002%). HPV-infected men reported use of poppers (22%) and sexual performance-enhancing drugs (10%). Median time to clearance was 6.3 months (IQR=5.9-12.1). Overall, recent drug use (of any type) was not associated with oral HPV clearance. Recent cocaine use was associated with reduced oral HPV clearance (HR=0.60, 95% CI=0.41-0.89). In multivariable analysis, recent cocaine use remained strongly associated with reduced oral HPV clearance (aHR=0.60, 95% CI 0.41-0.88). Results were similar when restricted to only HIV-infected participants within any one of the CD4 T cell strata (≥500, <500 cells/μl).

**Conclusions:** This study suggests that cocaine use may reduce clearance of oral HPV infection. Other types of recreational drug use did not influence oral HPV clearance.

## Background and rationale

Persistent oral human papillomavirus (HPV) infection increases risk for oropharyngeal squamous cell carcinoma (OPSCC), but risk factors for oral HPV persistence are poorly understood.[19] Tobacco, alcohol and recreational drugs such as marijuana, cocaine, amphetamines and opiates have immunomodulatory effects,[83-87] but their effect on oral HPV natural history is under-explored.

There is some evidence that cigarette smoking may increase risk for persistent oral HPV,[14,15,37,88] as it does for cervical HPV.[89] A study of the 6-month natural history of oral HPV in women found that oral HPV infections detected at baseline were more likely to be detected 6 months later among current smokers (adjusted odds ratio [OR]=8.0; 95% CI=1.3-53).[14] Similarly, a case-control study found that women cigarette smokers had a higher risk of persistent oral HPV, as compared to non-smokers ($\chi^2$ test, p=0.02; odds difference=2.29, 95% CI=1.18-4.44).[88] A 7-year cohort study in men found that smoking increased the risk that oral HPV infection would persist for at least 6 months (adjusted OR 1.92; 95% CI=1.05-3.50).[37] However, there have been contradicting reports that smoking has no effect on oral HPV natural history in men.[90,91] Additionally, questions remain about whether HPV clearance and persistence can be affected by the intensity and frequency of tobacco use, or simultaneous use of other substances. Interestingly, in a study of oral HPV natural history in a drug rehabilitation community where members were required to quit cigarette and recreational drug use, complete 1-year clearance of oral HPV infection among study participants was suggested to be due in part to smoking cessation and adoption of a healthier lifestyle.[34]

The effect of active recreational drug use on oral HPV natural history has not been previously studied, and data on the effects of alcohol are scant.[15] Laboratory and animal studies have found that alcohol, marijuana, crack, cocaine, amphetamines, opiates and a variety of inhalants have immunomodulatory effects, usually depressing but sometimes enhancing immune response.[83-85] These drugs can also decrease host response to microorganisms and enhance susceptibility to infection.[83-85] Tobacco, alcohol and recreational drug use are common in individuals with or at risk for HIV,[92-100] and have been linked to altered immune status, despite ART or HAART use.[92-95,101,102] There is growing evidence that certain drugs including cocaine, opiates, marijuana, alcohol and methamphetamine increase HIV infection and replication *in vitro* and in animal models, suggesting the possibility of additive or synergistic effects of drug use and HIV on immune function.[85,103-106] Initial studies suggest HIV may be associated with reduced clearance, possibly due to HIV-related immunosuppression, but further research is needed as results have been mixed.[42,107,108]

Recreational drugs may also impact oral HPV infection directly. Alcohol and cigarettes both contain carcinogens and mutagens,[109-111] and other drugs such as crack cocaine have been shown to cause nuclear alterations in oral mucosal cells.[112-114] Since the oral mucosa is the first site of exposure for many recreational drugs, it is plausible that these irritants and toxins may limit oral HPV clearance, but this hypothesis has not yet been investigated. The goal of this study was to explore the association of recreational drug use with oral HPV clearance in a U.S.-based cohort study of HIV-infected and HIV-uninfected adults.

## Methods

*Study design and study population*

From 2010 to 2014, 1,666 participants were enrolled in the "Persistence of Oral Papillomavirus Study" (POPS), a longitudinal study for studying oral HPV natural history. POPS was nested within two ongoing observational studies of men and women with or at risk for HIV: the Multicenter AIDS Cohort Study (MACS) and the Women's Interagency HIV Study (WIHS). POPS participants were enrolled at 6 study centers: Baltimore (MACS), Los Angeles (MACS), Pittsburgh (MACS), Brooklyn (WIHS), Bronx (WIHS), and Chicago (MACS and WIHS).[65-67] Each study center's Institutional Review Board approved the study. All participants provided written informed consent.

*Data collection*

At each semi-annual study visit, a 30-second Scope® oral rinse and gargle sample was collected to obtain oral exfoliated cells for HPV testing. A blood sample was also obtained, from which HIV status and CD4 T cell count were determined.

Participants completed a structured questionnaire about recreational drug use in the past 6 months.[115,116] Data were collected on recent use of:

- Alcohol
- Cigarettes
- Marijuana
- Crack
- Cocaine
- Heroin

- Amphetamines (e.g. speed, methamphetamines)
- Ecstasy/club drugs (e.g. ketamine, GHB)
- Speedball (cocaine and heroin or morphine injected together)
- "Poppers" or inhaled nitrites (assessed in men only)
- Sexual performance enhancing drugs (e.g. not prescribed for erectile dysfunction; assessed in men only)

At POPS enrollment, participants completed a supplemental questionnaire, which assessed oral health (i.e. amount of saliva). Questionnaires were administered via Computer-assisted Self-interviewing (CASI) in the MACS and were interview-administered in the WIHS. (See Appendices B and C for data collection tools)

*Oral HPV detection and genotyping*

Oral rinse samples were tested for 37 types of HPV DNA using PCR, followed by reverse line-blot hybridization, as described in detail in Chapter 3 Methods section.[68] Each sample was evaluated for the presence of high-risk/oncogenic types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66) and low-risk/non-oncogenic types (6, 11, 26, 38, 40, 42, 53, 54, 55, 61, 62, 64, 67, 68, 69, 70, 71, 72, 73, 81, 83, 84, 89, IS39), classified according to criteria from the International Agency for Research on Cancer.[7,69,70] Participants with any of these HPV types were considered HPV-infected. Prevalent oral HPV infection was defined as any type-specific oral HPV infection detected at study enrollment. Incident infection was defined as newly detected type-specific infection that was preceded by at least one HPV-negative oral rinse.

Time of oral HPV infection was defined as the visit of first type-specific oral HPV detection and clearance was defined as the visit of subsequent HPV-negative oral rinse. A secondary stricter definition of oral HPV clearance was also considered, requiring 2 consecutive HPV-negative oral rinses.

*Statistical methods*

Patient characteristics were summarized using descriptive statistics. Drug use prevalence was defined as the number of participants reporting use of each drug at the time of oral HPV detection out of 594 HPV-infected participants. Kaplan-Meier survival curves were used to plot the influence of drug use on time to clearance. Differences in survival curves were assessed using the log-rank test.

The relationship between use of each drug and type-specific oral HPV clearance was evaluated with unadjusted and adjusted WLW regression models,[72] accounting for within-participant clustering of HPV infections. There were few users of speedball and ecstasy/club drugs, so these 2 drug types were excluded from analyses of the effect of individual drugs on time to clearance but included in calculation of composite drug use variables (i.e. use of any drug, poly drug use).

Variables associated with time to clearance at $p<0.10$ in univariable (unadjusted) analysis were considered for inclusion in the multivariable (adjusted) model and were retained in the final model if statistically significant at $p<0.05$. Some variables (i.e. age) were included in multivariable analyses *a priori* based on prior evidence of being an important determinant for clearance of oral HPV infection. A number of potential confounders were considered, including HPV type and oral health. AIC, a standard

statistical test, was used to test the suitability of the models. The final multivariable model adjusted for biologic sex, HPV infection type (prevalent vs. incident), age (<45, 45-54, ≥55), HIV status, CD4 T cell count (≥500, <500 cells/μL), and oral health (saliva amount: normal/too little/too much).

In the primary analyses, recreational drug use exposure was defined as participant-reported use in the past 6 months (i.e. roughly, since the last study visit) and was modeled as a time-updated variable (i.e. time-dependent, allowing change of drug use status with each subsequent visit). Secondary analyses explored recency of drug use (i.e. drug use in the 6 months vs. past 12 months, to account for potential time lag in the effects of drug exposure, and because drug effects may persist even after drug cessation or with no recent use), drug use reported at time of oral HPV detection only (i.e. drug use as a time fixed variable, since drug exposure closer to the time of oral HPV infection may be more relevant to oral HPV natural history), and cumulative drug use reported over the course of oral HPV infection (to account for additive effects of drugs).

To test the dose-response relationship of drug exposure to HPV clearance, the frequency of drug use was stratified for analyses, with non-users in the lowest category and daily users in the highest category. For cigarettes and alcohol use, the intensity of use (i.e. packs smoked, number of drinks consumed per day) was assessed. Since poly drug use is common in the study population, the total number of drugs used (both including and excluding cigarette and alcohol use) was explored. Given the possibility that direct trauma to the oral mucosa may impact oral HPV clearance, the effect of any drugs smoked/snorted was also examined.

Hazard ratios (and 95% CIs) for HPV clearance were compared between infections in different drug use categories. Analyses were conducted with Stata12.[73] Statistical significance was defined as two-sided p-value<0.05.

## Results

*Participant characteristics*

Out of 1,666 participants, 594 HPV-infected participants with 1,358 type-specific oral HPV infections were identified. Half of participants (54%) were men, most were of Black race (55%), and the median age was 50 years (Table 4-1). Of the participants who were HIV-infected (72%), 81% were on current antiretroviral therapy (ART) and half (50%) had normal CD4 T cell count (≥500 cells cells/μL).

At time of first oral HPV detection, HPV-infected participants reported recent use of alcohol (65%), cigarettes (47%), marijuana (27%), crack (10%), cocaine (6%), heroin (2%), amphetamines (2%), ecstasy/club drugs (0.005%) and speedball (0.002%) (Figure 4-1). Most participants (86%) reported use of at least 1 drug type (excluding poppers and sexual performance-enhancing drugs). Of these participants, 47% reported concurrent use of more than 1 drug type. HPV-infected men reported use of poppers (22%) and sexual performance-enhancing drugs (10%) (Figure 4-1).

*Predictors of oral HPV clearance*

Predictors of oral HPV clearance were evaluated over 18,465 person-months of follow-up. Median time to clearance of oral HPV infection was 6.3 months (IQR: 5.9-12.1)

Overall, recent drug use (i.e. use of any drug type) was not significantly associated with clearance (HR=0.98, 95% CI=0.83-1.16) (Table 4-2). Recent cocaine use was associated with reduced oral HPV clearance (HR=0.60, 95% CI=0.41-0.89) (Table 4-2, Figure 4-2). While there was some suggestion cocaine use had a stronger effect in HIV-infected participants (HR=0.55, 95% CI=0.34-0.87) as compared to HIV-uninfected participants (HR=0.86, 95% CI=0.43-1.70), this difference was not statistically significant (p-interaction=0.30). The effect of cocaine on clearance was similar for oncogenic and non-oncogenic HPV types, and for both men and women. When considering any cocaine use in the past year, the association with reduced clearance was in the same direction but attenuated (HR=0.82, 95% CI=0.62-1.08).

Recent (past 6 months) cocaine use remained strongly associated with reduced oral HPV clearance (aHR=0.60, 95% CI=0.41-0.88), after adjusting for biologic sex, HPV infection type (prevalent vs. incident), age, HIV status, CD4 T cell count, and oral health (saliva amount: normal/too little/too much) (Table 4-3). Results were similar when restricted to only HIV-infected participants within any one of the CD4 T cell strata ($\geq$500, <500 cells/µl). In this adjusted model, male sex (aHR=0.79, 95% CI=0.69-0.91), prevalent infection type (aHR=0.43, 95% CI=0.37-0.49) and dry mouth (aHR=0.80, 95% CI= 0.65-0.98) remained associated with reduced oral HPV clearance.

No other drug types were associated with oral HPV clearance, when considering use in the past 6 months only, or any use in the past year. For each of the drug types analyzed, baseline drug use (reported at the time of oral HPV detection) was not a superior predictor of time to clearance, as compared to time-updated drug use. Cumulative drug use was not associated with oral HPV clearance. For cigarettes and

alcohol use, the intensity of use (i.e. packs smoked, number of drinks consumed per day) was assessed but found to not influence oral HPV clearance. When examining cigarette smoking categorized by smoking history, current smoking (HR=0.69, 95% CI=0.55-0.87) and past smoking (HR=0.75, 95% CI=0.57-0.97) appeared to be associated with reduced oral HPV clearance, as compared to never smoking, in women but not men (p-interaction=0.18). In an adjusted model, smoking was not associated with oral HPV clearance for either sex.

The frequency of use of marijuana, crack, poppers and sexual performance enhancing drugs was not significantly associated with time to clearance. There were too few cases of oral HPV clearance in cocaine, heroin and amphetamine users to examine risk of clearance by frequency of use.

Poly drug use (i.e. simultaneous use of more than one drug) was not associated with oral HPV clearance. When excluding alcohol and tobacco use, use of 2 or more drugs was significantly associated with reduced clearance (aHR=0.71, 95% CI=0.54-0.94), primarily due to the effect of cocaine use. When evaluating drugs by mode of use (e.g. any smoked/snorted/inhaled drugs), no associations with oral HPV clearance were observed. Comparing mode of use (i.e. snorted vs. injected) among cocaine users only was not possible because nearly all cocaine users snorted the drug.

## Discussion

To our knowledge, this is the first study to explore the association of recreational drug use with oral HPV clearance. While most drugs had no effect on oral HPV clearance, cocaine use appeared to reduce clearance.

Cocaine is a commonly used stimulant drug that blocks the reuptake of monoamine neurotransmitters (norepinephrine, serotonin and dopamine) in the brain.[117] It is taken recreationally for its intense euphoric effects. There are approximately 14 million to 21 million estimated past-year cocaine users globally and approximately 1.9 million past-month cocaine users in the U.S.[118,119] When snorted, cocaine is rapidly absorbed by mucous membranes and reaches systemic circulation within minutes.[120] Smaller cocaine particles can become trapped by tracheobronchial mucous and carried by epithelial cilia to the oropharynx, and then become detectable in saliva.[121]

Animal and human studies have shown that cocaine exposure can have broad immunosuppressive effects on leukocyte populations, including T cells, B cells and natural killer (NK) cells,[83,84,117,122] and may differentially stimulate certain T cell subsets.[102] In addition, cocaine can cause imbalances in cytokine production, reducing immune responsiveness to viral infections.[83-85,117] Given that a healthy immune response is required for HPV clearance,[46] the immunosuppressive effects of cocaine may explain why those reporting recent use experienced reduced clearance of infection. Direct damage to oral mucosal cells may also account for the delayed clearance.[123,124]

Cocaine use in the 6 months before oral HPV detection was more strongly associated with reduced oral HPV clearance than cocaine use in the 12-month period before HPV detection. We carefully considered the appropriate time frame for capturing the effects of cocaine use, especially since past research has suggested that cocaine cessation, in addition to both acute and chronic cocaine exposure, may result in immunosuppressive effects.[125] We found that cocaine use closer to the time of oral HPV detection had the greater impact on oral HPV natural history.

Given that the degree of immune suppression induced by cocaine exposure depends highly on dose and frequency of administration,[117] it may have been informative to evaluate whether frequency of cocaine use affected clearance, but we were limited by small sample size. Cocaine is sometimes used in "binges", especially as it has a relatively short-lasting effect and induces physical addiction. Therefore, additional study could provide helpful data on whether the intensity of cocaine usage corresponds to reduced oral HPV clearance in a dose-response fashion.

Previous studies have demonstrated that cocaine use increases HIV pathogenesis and has detrimental health effects in HIV-infected individuals, even amongst those who are ART-adherent.[85,103,126-128] We considered the possibility that the effect of cocaine use on reduced clearance was mediated by HIV-related immune factors. However, we found that the effects of cocaine were similar across CD4 T cell strata among HIV-infected individuals, suggesting that the effects of cocaine were independent of HIV-related immunosuppression. HAART use was also found to be unrelated to oral HPV clearance in this study population.

Poly drug use was common in this study population, as in other high-risk populations,[83] and so it was not possible to isolate the effects of cocaine alone on oral HPV natural history. Evidence of cocaine-mediated immunosuppression has been repeatedly demonstrated by laboratory studies of cocaine, isolated from the contaminating effects of other potentially immunomodulatory drugs.[84] This supports our hypothesis that cocaine-related immunosuppression may lead to reduced oral HPV clearance. Also, none of the other drugs studied had any significant impact on oral HPV

clearance so it is unlikely that the effects of cocaine observed are due to the effects of other drugs used concurrently.

In this study, crack use was not associated with oral HPV clearance. The reasons why cocaine, but not crack, was associated with oral HPV clearance are not clear. As crack is derived from powder cocaine (by mixing with a solvent and baking soda), the chemical composition of crack and cocaine are very similar and thus their differences are primarily in the route of administration.[129] In this study, cocaine was primarily snorted and crack was smoked. It is possible that this difference in drug delivery may have accounted for differences in effects, perhaps since snorted cocaine particles are more likely to cause micro-abrasions to oral mucosa. Differences in observed effects may also be due to solvents used in forming crack cocaine from powder cocaine, or contaminants/adulterants added to enhance drug effects or attenuate its side effects[129,130] Another potential explanation is that the heat used to smoke crack destroys the active drug, or leads to loss by vaporization; indeed, smoking is known to be a less efficient form of drug delivery, resulting in lower bioavailability than intranasal administration.[129,131]

This study found that, overall, smoking had no effect on oral HPV clearance. These results contrast with other studies that have found smoking to be a risk factor for oral HPV persistence.[14,37,88] This discrepancy may be due to differences in study population or in method of oral HPV detection and/or testing. While we used oral rinse to obtain exfoliated oral cells, 2 of the prior studies used Cytobrush® oral scrapings from the buccal mucosa for oral HPV testing.[37,88] Both of those studies also used a different testing platform and tested for 24 HPV types while we tested for an additional 13 HPV types. In

this study, the data suggested that smoking might reduce clearance in women but not men. This is consistent with a previous study in this same cohort at 3 years of follow-up, which found that HIV-infected women smokers had reduced oral HPV clearance;[15] however, differences by HIV status and sex did not reach statistical significance in this study.

It is unclear why alcohol, cigarettes and other drugs with documented immune effects (e.g. heroin, amphetamines, marijuana),[83,84] did not influence oral HPV clearance. Self-reported drug use may not reflect biological exposure, and thus, future studies may consider measuring objective markers of drug exposure (i.e. serum cotinine), when possible.[132,133]

A novel finding in this study was the association of dry mouth with reduced oral HPV clearance. Dry mouth has been associated with use of recreational drugs including cocaine and other stimulants, various prescription medications and polypharmacy, older age, menopause, and a number of medical conditions.[133-136] Saliva has various antimicrobial components including defensins which are antiviral agents,[137] so it is plausible that a reduction in saliva may decrease oral immune defenses, increasing host susceptibility to oral HPV persistence. There is prior evidence that other measures of poor oral health, including tooth loss or infrequent tooth brushing, are associated with oral HPV infection and OPSCC.[3,5,138] A case-control study found that women with persistent oral HPV infection have different salivary composition, including higher salivary IgG and lysozyme, than women without oral HPV.[88] Taken together, this evidence supports the hypothesis that oral health, and possibly dry mouth, may play a role in oral HPV infection. However, it remains unclear whether dry mouth, and other

measures of oral health, affects the composition of saliva or just the amount of saliva. Poor oral health is a common health concern among alcohol/drug-abusers so it is possible that reduced clearance results from other unmeasured drug-associated factors.[120,139,140] Saliva amount in this study was self-reported and although questionnaire-based assessment of dry mouth is a commonly used method for clinical diagnosis of dry mouth (i.e. xerostomia), objective measurement of salivary gland function and saliva properties may clarify the role of local immunity in oral HPV infection. Also, as interest in oral immunity has increased, validated tools such as the Xerostomia Inventory have been developed for more reliable subjective assessments of dry mouth.[136]

Our findings should be interpreted within the limitations of the study. Drug use and oral health were self-reported and thus subject to recall and other biases. Cocaine use was relatively uncommon so analyses stratified by frequency of cocaine use could not be done. Strengths of this study included the longitudinal follow-up, high participant retention and large number of common recreational drug types assessed. The use of rinse and gargle samples enabled collection of cells from the back of the throat and may have yielded more HPV DNA than studies that used oral swabs or brushes.

## Conclusion

This study is the first to report that cocaine use may be an important risk factor for oral HPV persistence. These initial findings warrant confirmation from other studies and populations. Further examination could increase understanding of the effects of frequency and intensity of cocaine use, as well as mechanisms by which cocaine use might reduce oral HPV clearance.

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**Table 4-1.** Participant characteristics at time of first oral HPV detection

| Participant characteristic[1] | No. | % |
|---|---|---|
| **Sex** | | |
| Women (WIHS) | 274 | 46% |
| Men (MACS) | 320 | 54% |
| **Infection type[2]** | | |
| Incident only | 189 | 32% |
| Any Prevalent | 405 | 68% |
| **Age (year)** | | |
| <45 | 162 | 27% |
| 45-54 | 258 | 43% |
| ≥55 | 174 | 29% |
| **Race/Ethnicity** | | |
| White, non-Hispanic | 186 | 31% |
| Black, non-Hispanic | 328 | 55% |
| Other race or Hispanic | 80 | 14% |
| **Study site[3]** | | |
| Baltimore, Maryland | 88 | 15% |
| Bronx, New York | 93 | 16% |
| Brooklyn, New York | 84 | 14% |
| Chicago, Illinois | 202 | 34% |
| Los Angeles, California | 24 | 4% |
| Pittsburgh, Pennsylvania | 103 | 17% |
| **HIV status** | | |
| Uninfected | 168 | 28% |
| Infected | 426 | 72% |
| **Current CD4 T cell count (cells/uL)** | | |
| ≥500 | 212 | 50% |
| <500 | 214 | 50% |
| **Current HIV RNA viral load (copies/mL)** | | |
| <200 | 286 | 68% |
| 200-19,999 | 78 | 19% |
| ≥20,000 | 57 | 14% |
| **Current ART use** | | |
| No | 83 | 20% |
| Yes | 343 | 81% |
| **Amount of saliva (self-reported)** | | |
| Don't notice/normal | 421 | 77% |
| Too little/dry mouth | 82 | 15% |
| Too much | 46 | 8% |

[1] All participant characteristics are from the time of oral HPV detection, with the exception of amount of saliva, which was reported at time of POPS enrollment.

[2] Prevalent infection was defined as oral HPV detected at POPS enrollment. Incident infection was defined as newly detected oral HPV preceded by at least one HPV-negative oral rinse. Some participants had prevalent infection, followed by incident infection of another HPV type that was included in the analysis.
[3] Baltimore, Los Angeles and Pittsburgh study sites enrolled men only (MACS). Bronx and Brooklyn study sites enrolled women only (WIHS). Chicago study site enrolled both men and women.

**Table 4-2.** Unadjusted associations of recent drug use, demographic and health factors with time to clearance among 594 participants with 1,358 oral HPV infections

| Participant characteristic[1] | HR (95% CI) |
|---|---|
| **Recent drug use[2]** | |
| Alcohol | 1.00 (0.87-1.15) |
| Cigarettes | 1.00 (0.87-1.16) |
| Marijuana | 0.98 (0.83-1.15) |
| Crack | 0.99 (0.79-1.23) |
| Cocaine | **0.60 (0.41-0.89)** |
| Heroin | 0.81 (0.55-1.19) |
| Amphetamines | 0.74 (0.38-1.43) |
| Poppers (men only)[3] | 1.04 (0.82-1.32) |
| Sexual performance-enhancing drugs (men only)[4] | 0.75 (0.54-1.04) |
| Any drug use[5] | 0.98 (0.83-1.16) |
| **Sex** | |
| Women (WIHS) | Ref |
| Men (MACS) | **0.79 (0.69-0.91)** |
| **Infection type** | |
| Incident | Ref |
| Prevalent | **0.43 (0.37-0.49)** |
| **Age (year)** | |
| <45 | Ref |
| 45-54 | 0.93 (0.79-1.10) |
| ≥55 | **0.79 (0.65-0.95)** |
| | **p-trend=0.01** |
| **HIV status and CD4 cell count (cells/uL)** | |
| HIV-uninfected | Ref |
| HIV-infected, CD4≥500 | 0.98 (0.82-1.16) |
| HIV-infected, CD4<500 | 0.89 (0.74-1.07) |
| | p-trend=0.18 |
| **Amount of saliva (self-reported)** | |
| Don't notice | Ref |
| Too little/dry mouth | **0.77 (0.62-0.96)** |
| Too much | 1.04 (0.82-1.34) |

[1] All participant characteristics are from the time of oral HPV detection, with the exception of amount of saliva, which was reported at time of POPS enrollment

[2] Drug use was considered a time-updated variable (i.e. drug use status reported at each visit during oral HPV infection was applied to the WLW model, and was allowed to change if the participant started/stopped drug use). If a participant missed a visit, the last reported drug use status was used in the analysis.

[3] Poppers were defined as nitrite inhalants such as amyl, butyl or isopropyl nitrites.
[4] Sexual performance-enhancing drugs were defined as drugs other than those prescribed by a medical provider for a diagnosed erectile dysfunction.
[5] Any drug used includes use of alcohol, cigarettes, marijuana, crack, cocaine, heroin, amphetamines, speedball, and ecstasy/club drugs (excludes poppers and sexual performance-enhancing drugs, which were only assessed in men).

**Table 4-3.** Multivariable risk factors for oral HPV clearance among 594 participants with 1,358 oral HPV infections

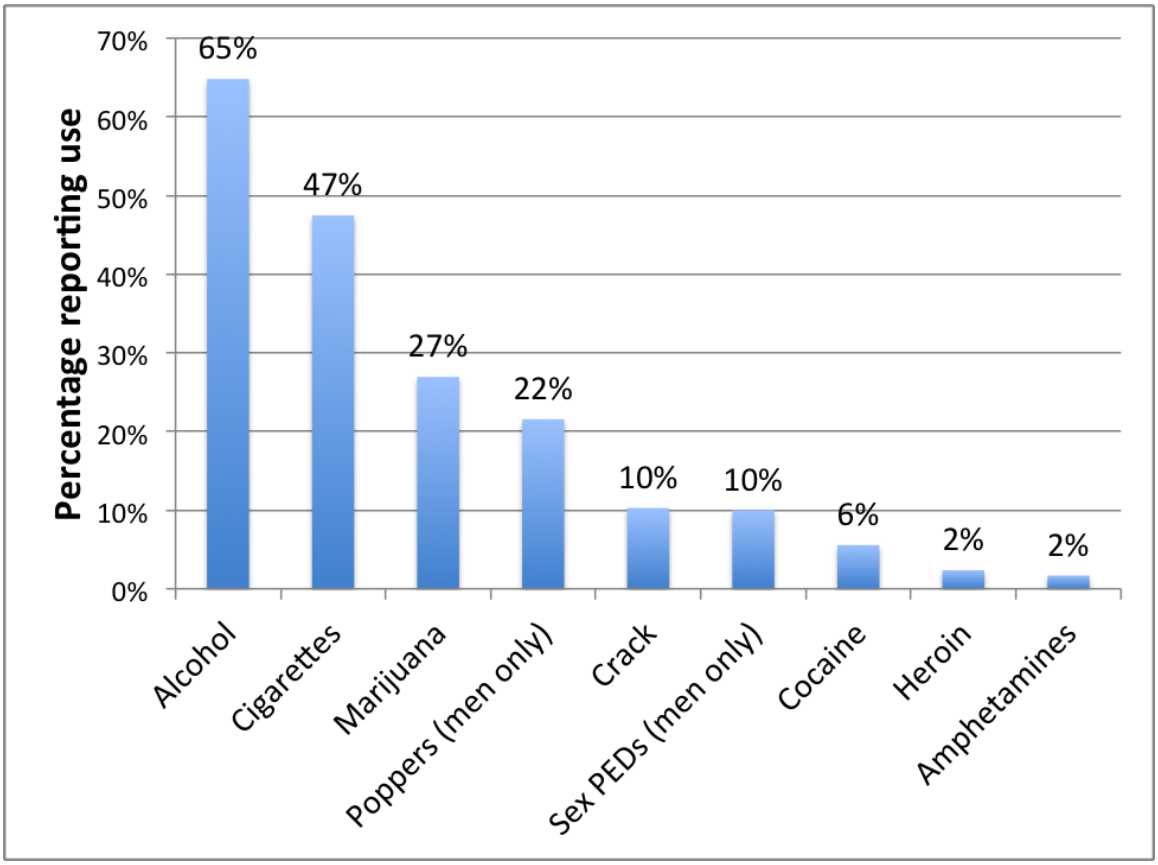
| Participant characteristic[1] | aHR (95% CI) |
|---|---|
| **Recent cocaine use[2]** | |
| No | Ref |
| Yes | **0.60 (0.41-0.88)** |
| **Sex** | |
| Women (WIHS) | Ref |
| Men (MACS) | **0.79 (0.69-0.91)** |
| **Infection type** | |
| Incident | Ref |
| Prevalent | **0.43 (0.37-0.49)** |
| **Age (year)** | |
| <45 | Ref |
| 45-54 | 0.96 (0.81-1.14) |
| ≥55 | 0.93 (0.77-1.11) |
| | p-trend=0.42 |
| **HIV status and CD4 cell count (cells/uL)** | |
| HIV negative | Ref |
| HIV positive, CD4≥500 | 1.00 (0.84-1.19) |
| HIV positive, CD4<500 | 0.87 (0.73-1.04) |
| | p-trend=0.10 |
| **Amount of saliva (self-reported)** | |
| Don't notice | Ref |
| Too little/dry mouth | **0.80 (0.65-0.98)** |
| Too much | 1.09 (0.84-1.41) |

aHR=adjusted hazard ratio

[1] All participant characteristics are from the time of oral HPV detection, with the exception of amount of saliva, which was reported at time of POPS enrollment

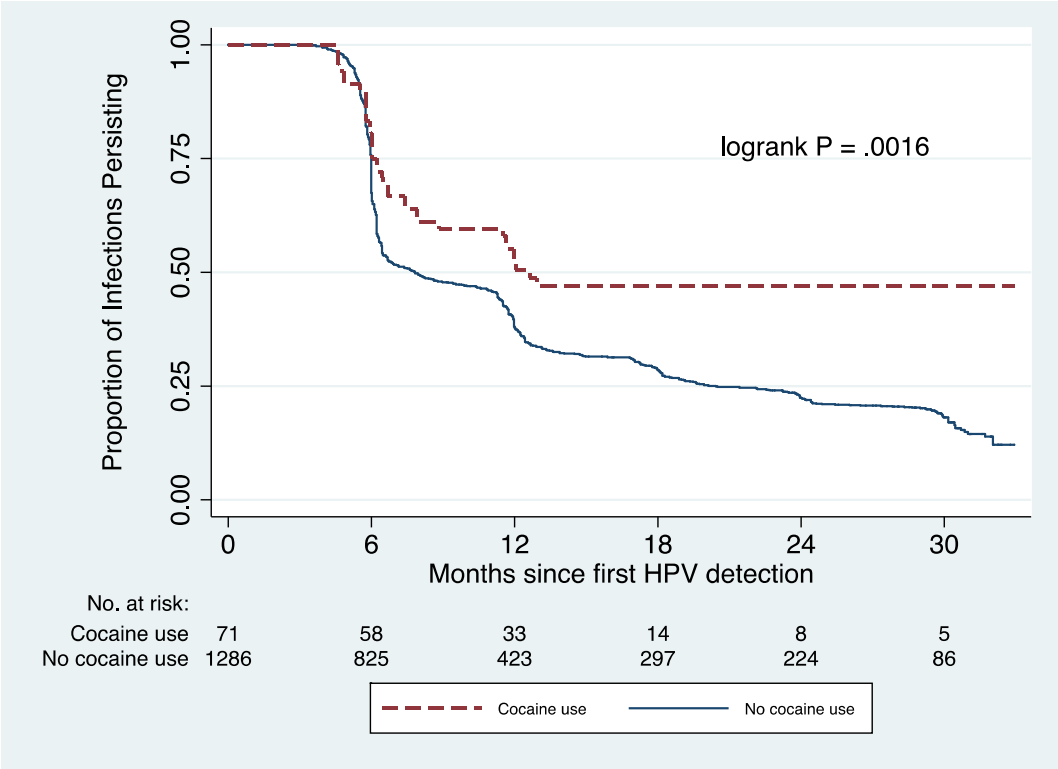
[2] Cocaine use was considered a time-updated variable (i.e. cocaine use status reported at each visit during oral HPV infection was applied to the WLW model, and was allowed to change if the participant started/stopped cocaine use). If a participant missed a visit, the last reported cocaine use status was used in the analysis.

**Figure 4-1.** Prevalence of drug use at time of first oral HPV detection




Sex PEDs = sexual performance enhancing drugs
Drug use was assessed in 594 HPV-infected participants, with the exception of poppers use and sex PED use, which were assessed in HPV-infected men only (n=320).
Not shown in figure: ecstasy/club drugs (0.005%) and speedball (0.002%).

**Figure 4-2.** Time to oral HPV clearance in 594 participants with 1,358 oral HPV infections, by recent cocaine use



# Chapter 5. The association of medication use with clearance or persistence of oral HPV infection


## Abstract

**Background:** With increasing lifespan of patients with HIV, the number of HIV-infected individuals taking multiple medications for comorbidities is increasing. While there is accumulating evidence on the immunomodulatory effects of medications for chronic conditions, the impact of these medications on oral HPV natural history has not been previously studied. Given that prescription medication use is a common exposure, and potential risk factor for oral HPV persistence, we conducted a study to investigate whether medication use may impact oral HPV natural history.

**Methods:** From 2010 to 2014, Scope® oral rinse and gargle samples were collected from 1,666 participants semi-annually. Samples were tested for 37 types of oral HPV DNA using PCR with PGMY primers followed by reverse line blot hybridization. At each visit, data were collected on recent (past 6 months) use of medications, including: antiasthmatics, antidepressants, antihypertensives, antipsychotics, anxiolytics and sedatives, cholesterol-lowering medications, diabetes medications, hormones, non-steroidal anti-inflammatory drugs (NSAIDs) and erectile dysfunction medications. The relationship between medication use and oral HPV clearance was evaluated with Wei-Lin-Weissfeld regression models, accounting for within-participant clustering of HPV infections. The final multivariable model adjusted for biologic sex, HPV infection type

(prevalent vs. incident), age (<45, 45-54, ≥55), HIV status and CD4 T cell count (≥500, <500 cells/μL).

**Results:** A total of 1,358 type specific oral HPV infections in 594 participants were detected and followed for 18,465 person-months. In unadjusted analyses, oral HPV clearance was significantly reduced in those with recent use of antipsychotics (HR=0.75, 95% CI=0.57-0.99), anxiolytics/sedatives (HR=0.78, 95% CI=0.63-0.96) and antidepressants (HR=0.82, 95% CI=0.67-0.999). Among users of antipsychotics, there appeared to be effect modification by HIV status, with reduced clearance in HIV-infected participants (HR=0.75, 95%: CI 0.57-0.99), but not HIV-uninfected participants (p-interaction=0.009). Use of erectile dysfunction medications was marginally associated with reduced oral HPV clearance (HR=0.77, 95% CI=0.58-1.01). No other medication type was significantly associated with oral HPV clearance. After adjusting for the effects of sex, infection type (incident vs. prevalent), age, HIV status and CD4 T cell count, antipsychotic use remained significantly associated with reduced oral HPV clearance overall (aHR=0.75, 95% CI=0.57-0.99), and when restricted to only HIV-infected participants (aHR=0.66, 95% CI=0.48-0.90). Anxiolytics/sedatives, antidepressants and erectile dysfunction medications remained associated with reduced clearance but these associations were not statistically significant.

**Conclusions:** Participants using antipsychotic medications had significantly reduced oral HPV clearance, with potential differential effects by HIV status. Antidepressants and anxiolytics/sedatives were also associated with reduced oral HPV clearance, especially

when taken in combination. Each of these medications are prescribed for conditions that have immunomodulating effects, so characteristics of the illness may partially contribute to reduced oral HPV clearance.

## Background and rationale

Antiretroviral therapies (ART) have significantly increased survival of HIV-infected individuals, transforming HIV into a chronic and manageable condition.[141] As people with HIV live longer, there has been an increase in comorbidities and use of medications to treat these conditions.[142,143] While there is growing evidence on the immunomodulatory effects of medications for chronic conditions, the impact of these medications on oral HPV natural history is unknown. Given that prescription medication use is a common exposure in individuals with or at risk for HIV,[144] a population with high oral HPV prevalence, we conducted a study to investigate whether medication use influences oral HPV clearance.

Individuals with HIV suffer from a higher number of comorbidities from a younger age and are at greater risk of age-related comorbidities, as compared to HIV-uninfected individuals.[141,143,145,146] Studies suggest that HIV infection is associated with poorer health, through effects on inflammation and interaction with other risk factors common in high-risk populations such as prolonged substance use.[141,144] In one observational study of HIV-infected individuals ≥50 years of age in the UK, 84% of participants reported having ≥1 comorbid conditions and 61% reported >2 comorbid conditions.[147] The most commonly reported conditions were high cholesterol, sexual dysfunction, hypertension and depression. Indeed, as AIDS-defining illnesses are becoming more rare in individuals with ART-suppressed HIV, HIV-associated non-AIDS conditions are increasingly common.[141,148] These include conditions associated with advancing age and chronic inflammation including but not limited to diabetes mellitus, cardiovascular diseases, liver disease, and neurocognitive decline.[141,148]

For each comorbidity diagnosed, more than one type of medication may be prescribed for treatment or management of the condition.[145] As a result, HIV-infected individuals are commonly taking multiple medications, in addition to ART, 2 to 3 decades before HIV-uninfected individuals.[145] In a cross-sectional study of HIV-infected patients in the Southern U.S., 93% were receiving non-ART medications including antihypertensives (43%), lipid-lowering agents (34%) and antidepressants/antipsychotics/anxiolytics (34%), and the median number of medications per patient was 8 (IQR: 6-11).[149] Concurrent use of multiple medication types is more common among older HIV-infected patients, as compared to the elderly in the general population.[146] In a study of 130 HIV-infected patients >50 years of age, more than half took five or more different medications, in addition to ART, over a 12-month study period.[146] As HIV-infected individuals age, the number of comorbidities and medications prescribed to treat these comorbidities is increasing.[142]

Several commonly prescribed medication types have been shown to have immunomodulatory effects, either as part of their primary therapeutic activity or as a side effect, including: antidepressants,[150-153] antipsychotics,[154-157] sedatives/anxiolytics,[158-165] anti-asthmatics,[166-173] antihypertensives,[174] cholesterol-lowering medications[174-181] hormonal treatments,[182-186] erectile dysfunction drugs,[187] and diabetes medications.[175,188-193] While it is plausible that medication use may impact oral HPV natural history, this has not been previously investigated. As the MACS and WIHS are aging cohorts with a high prevalence of oral HPV, multimorbidity and polypharmacy, this presents an opportunity for studying the impact of medication use on oral HPV natural history in a high-risk population.

## Methods

*Study design and study population*

From 2010 to 2014, 1,666 participants were enrolled in the "Persistence of Oral Papillomavirus Study" (POPS), a longitudinal study for studying oral HPV natural history. POPS was nested within two ongoing observational studies of men and women with or at risk for HIV: the Multicenter AIDS Cohort Study (MACS) and the Women's Interagency HIV Study (WIHS). POPS participants were enrolled at 6 study centers: Baltimore (MACS), Los Angeles (MACS), Pittsburgh (MACS), Brooklyn (WIHS), Bronx (WIHS), and Chicago (MACS and WIHS).[65-67] Each study center's Institutional Review Board approved the study. All participants provided written informed consent.

*Data collection*

At each semi-annual study visit, a 30-second Scope® oral rinse and gargle sample was collected to obtain oral exfoliated cells for HPV testing. A blood sample was also obtained, from which HIV status and CD4 T cell count were determined.

Participants completed a structured questionnaire about their medication use in the past 6 months.[115,116] Data were collected on recent use of:

- Antiasthmatics (steroidal and non-steroidal)
    - Asthma medications were classified as steroidal anti-asthmatics, which suppress airway inflammation; and non-steroidal anti-asthmatics, or bronchodilators, which relax airway smooth muscle. These medications are also used to treat chronic obstructive pulmonary disease (COPD).

- Antidepressants
- Antihypertensives
- Antipsychotics – used to treat schizophrenia, bipolar disorder and related psychotic disorders
- Anxiolytics/sedatives – used to treat a variety of disorders including, but not limited to: anxiety, sleeping disorders, epilepsy, alcohol withdrawal and muscle spasms. For the purposes of this study, anxiolytics and sedatives are considered a single medication group.
- Cholesterol-lowering medications
- Diabetes medications
- Hormones, including hormone replacement therapy (e.g. estrogen, progesterone) or hormones used to treat thyroid disorders. This excludes hormones taken only to prevent pregnancy but that may be used for hormone replacement therapy including birth control pills, Norplant® or NuvaRing®.
- Non-steroidal anti-inflammatory drugs (NSAIDs)
- Erectile dysfunction medications (men only) – used to treat physician-diagnosed erectile dysfunction

At POPS enrollment, participants completed a supplemental questionnaire, which assessed oral health (i.e. amount of saliva). Questionnaires were administered via Computer-assisted Self-interviewing (CASI) in the MACS and were interview-administered in the WIHS. (See Appendices D and E for data collection tools) See Appendix F for details of medication classifications and examples of medication types included in each medication class.

*Oral HPV detection and genotyping*

Oral rinse samples were tested for 37 types of HPV DNA using PCR, followed by reverse line-blot hybridization, as described in detail in Chapter 3 Methods section.[68] Each sample was evaluated for the presence of high-risk/oncogenic types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66) and low-risk/non-oncogenic types (6, 11, 26, 38, 40, 42, 53, 54, 55, 61, 62, 64, 67, 68, 69, 70, 71, 72, 73, 81, 83, 84, 89, IS39), classified according to criteria from the International Agency for Research on Cancer.[7,69,70] Participants with any of these HPV types were considered HPV-infected. Prevalent oral HPV infection was defined as any type-specific oral HPV infection detected at study enrollment. Incident infection was defined as newly detected type-specific infection that was preceded by at least one HPV-negative oral rinse.

Clearance was defined as two consecutive HPV-negative oral rinses, with time of clearance as the visit of the first negative oral rinse sample. A secondary less conservative definition of clearance was considered, requiring only one HPV-negative oral rinse sample.

*Statistical analyses*

Participant characteristics were summarized using descriptive statistics. Medication use prevalence was defined as the number of participants reporting use of each medication at time of oral HPV detection, out of 594 HPV-infected participants. For medications that are often prescribed concurrently (e.g. cholesterol-lowering and hypertension medications), or that are used to treat illnesses with similar clinical

presentations (e.g. antipsychotic and antidepressant medications), the prevalence of concomitant medication use was calculated in this same way.

The analysis included 1,358 type-specific oral HPV infections among 594 participants (some participants were infected with multiple HPV types at the same visit and medication use status reported at that visit was applied to clearance analysis of all those infections). The effect of each medication type on oral HPV clearance was modeled using WLW models, accounting for within-participant clustering of HPV infections.[72] First, the effect of each medication was considered in separate univariable models. Then common concomitant medication pairs were also modeled separately to assess whether use of both medications together was associated with time to clearance, as compared to using only one of these medications or none of these medications. For medications with statistically significant associations with time to clearance, the number of these medications used concurrently was evaluated to determine whether increasing medication use resulted in cumulative effects on clearance.

Variables associated with time to clearance at $p<0.10$ in univariable (unadjusted) analysis were considered for inclusion in the multivariable (adjusted) model and were retained in the final model if statistically significant at $p<0.05$. Some variables (i.e. age) were included in multivariable analyses *a priori* based on prior evidence of being important determinants for clearance of oral HPV infection. A number of potential confounders were considered, including HPV type and oral health. AIC, a standard statistical test, was used to test the suitability of the models. The final multivariable model adjusted for biologic sex, HPV infection type (prevalent vs. incident), age (<45, 45-54, ≥55), HIV status, and CD4 T cell count (≥500, <500 cells/μL). Stata 12 statistical

software package was used for statistical analyses.[73] Statistical significance was defined by a two-sided p-value <0.05.

## Results

*Participant characteristics*

The study population in this analysis is the same as the one in the analysis of recreational drug use (Chapter 4, Table 4-1). Participant characteristics are reproduced below for clarity (Table 5-1). Out of 1,666 participants, 594 HPV-infected participants with 1,358 type-specific oral HPV infections were identified. Half of participants (54%) were men, most were of Black race (55%), and median age was 50 years (Table 5-1). Of the participants who were HIV-infected (72%), 81% were on current antiretroviral therapy (ART) and half (50%) had normal CD4 T cell count (≥500 cells cells/μL).

Among 594 HPV-infected study participants, the most commonly used prescription drugs were hypertension medications (39% of participants), NSAIDs (39%), cholesterol-lowering drugs (27%), antidepressants (25%) and anxiolytics/sedatives (21%) (Figure 5-1). At time of oral HPV detection, 84% of participants reported use of at least 1 medication (excluding erectile dysfunction medications). Among men only, 17% reported use of erectile dysfunction drugs prescribed by a physician.

At time of oral HPV detection, 34% of participants reported concurrent use of more than 1 medication. The most commonly used medication pairs were: cholesterol-lowering and hypertension medications (15% of all study participants), hypertension and diabetes medications (9%), cholesterol-lowering and diabetes medications (7%) and antipsychotics and antidepressants (7%) (Figure 5-2).

*Effect of medication use on time to oral HPV clearance*

Oral HPV clearance was evaluated over 18,465 person-months of follow-up. Median time to clearance of oral HPV infection was 6.3 months (IQR: 5.9-12.1)

In unadjusted analyses, reduced oral HPV clearance was significantly associated with recent use of antipsychotics (HR=0.75, 95% CI=0.57-0.99), anxiolytics/sedatives (HR=0.78, 95% CI=0.63-0.96) and antidepressants (HR=0.82, 95% CI=0.67-0.999) (Table 5-2). Use of erectile dysfunction medications was marginally associated with reduced oral HPV clearance (HR=0.77, 95% CI=0.58-1.01). No other medication type was significantly associated with oral HPV clearance.

Among users of antipsychotics, there appeared to be effect modification by HIV status, with reduced clearance in HIV-infected participants (HR=0.67, 95% CI=0.49-0.91), but not HIV-uninfected participants (p-interaction=0.009) (Table 5-2). Although statistically significant, the numbers of HIV-uninfected participants reporting use of antipsychotics at time of oral HPV detection was small (n=10), which limits interpretation of this possible interaction by HIV status. Use of anxiolytics/sedatives and antidepressants were more common among HIV-uninfected than HIV-infected participants and the associations of these medications did not differ by HIV status (p-interaction=0.99 and 0.81, respectively). The association of non-steroidal antiasthmatics with oral HPV clearance appeared to differ by HIV status (p-interaction=0.012), but the association with reduced clearance in HIV-infected participants was non-significant (p=0.73).

After adjusting for the effects of sex, infection type (prevalent vs. incident), age, HIV status and CD4 T cell count, antipsychotic use remained significantly associated with reduced oral HPV clearance overall (aHR=0.75, 95% CI=0.57-0.99), and when restricted to only HIV-infected participants (aHR=0.66, 95% CI=0.48-0.90) (Table 5-3). Anxiolytics/sedatives (aHR=0.89, 95% CI=0.73-1.07), antidepressants (aHR=0.89, 95% CI=0.71-1.11) and erectile dysfunction medications (aHR=0.78, 95% CI=0.59-1.05) were associated with reduced clearance but these associations were not statistically significant.

*Concomitant medication use*

Of the participants using antipsychotics, 67% also reported use of antidepressants and 29% also reported use of anxiolytics/sedatives at time of oral HPV detection. When evaluating concurrent use of these 3 medications in unadjusted analyses, participants who reported recent use of all 3 medications had the greatest reduction in clearance (HR=0.57, 95% CI=0.35-0.94); followed by participants who used any 2 of these medications (HR=0.73, 95% CI=0.55-0.96) or only 1 of these three medications (HR=0.83, 95% CI=0.69-1.00), as compared to participants who used none of these medications (p-trend=0.002) (Figure 5-3). In a fully adjusted model, concomitant use of antipsychotics, antidepressants, and anxiolytics/sedatives remained marginally associated with reduced clearance, with greater number of medications used being more strongly associated (p-trend=0.04). When restricting the analyses to use of only antipsychotics, only antidepressants or only anxiolytics/sedatives, each medication remained associated with reduced clearance, but no longer reached statistical significance. Concomitant use of

other medications (i.e. commonly paired medications) was not significantly associated with oral HPV clearance.

## Discussion

While most medications did not affect oral HPV clearance, some potentially immunomodulatory medications decreased oral HPV clearance. This effect was most notable for anti-psychotic use. In addition to antipsychotics, antidepressants and anxiolytics/sedatives appeared to affect oral HPV clearance, especially when these medications were taken in combination.

*Association of antipsychotic medications and oral HPV clearance*

The immunomodulatory effects of antipsychotics, which are used for treatment of schizophrenia or bipolar disorder, have been consistently reported in *in vivo*, *in vitro* and *ex vivo* studies.[154-157] According to a recent meta-analysis, which included 23 studies (762 subjects being treated for schizophrenia), antipsychotic medications have anti-inflammatory immune effects, characterized by increased plasma IL-12 and soluble IL-2 receptor (sIL2R), and decreased IL-1β and IFN-γ.[157] However, the nature of cytokine alterations (i.e. which cytokines are down-regulated and which are up-regulated) may vary depending on patient characteristics. [154,157] An independent, more restrictive meta-analysis of 180 first-episode psychosis subjects across 5 clinical cohorts, found that after controlling for age and gender, as well as BMI and smoking when possible, antipsychotics had some pro-inflammatory effects, characterized by increased IL-15, in addition to anti-inflammatory effects.[154] Therefore, while there is substantial evidence

that antipsychotics induce immunomodulation, it is not clear whether the overall effect is anti-inflammatory or pro-inflammatory, limiting our ability to speculate on the mechanism by which antipsychotic medications may reduce oral HPV clearance.

It is also possible that reduced oral HPV clearance is associated with the immunomodulatory effects of psychotic disorder, either in addition to or independent of any association with the medications used to treat these disorders. Schizophrenia and bipolar disorder are commonly diagnosed psychotic conditions. Both of these illnesses are characterized by dysregulation of the immune response and upregulation of pro-inflammatory cytokines (i.e. lean towards a Th1-like cytokine profile). [156,194 195-197] The efficacy of antipsychotic medications is presumably due to their ability to normalize the levels of cytokines that are elevated in patients with psychosis. However, not all cytokines are affected by antipsychotics. TNF-α concentrations are consistently higher in patients with schizophrenia[156,198,199] and bipolar disorder[200], as compared to healthy controls, and according to a recent meta-analysis, peripheral TNF-α concentrations do not change upon treatment with antipsychotics.[157] Furthermore, another meta-analysis suggested that TNF-α, along with IL-12, IFN-γ and sIL-2R, may be trait markers for schizophrenia as levels remain high even after use of antipsychotics.[156] If TNF-α plays a role in oral HPV natural history, as suggested by the results of our study looking at the association of serum cytokines with oral HPV clearance (Chapter 3), then this lack of effect of antipsychotics on TNF-α may help explain why reduced clearance is observed in antipsychotics users, even though these medications supposedly normalize cytokine levels. This also supports the notion that reduced oral HPV clearance is due to the

immunomodulatory effects of psychotic disorder, and not the medications prescribed to treat these disorders.

The association of antipsychotics with reduced clearance could also be explained by biological or behavioral factors unique to individuals with psychotic disorders. Furthermore, the etiology of psychosis is heterogeneous as it is a symptom of multiple mental health illnesses.[201] For example, atypical antipsychotics are sometimes used for treatment of patients with severe or treatment-resistant depressive disorders.[202] Since information on subtypes of psychotic disorders was not available, heterogeneity in study participants taking antipsychotics could not be assessed. Variations in antipsychotic medication types prescribed (i.e. differences in brand, dose or duration) or differences in stage of illness (i.e. first-episode psychosis vs. chronic disease) could also have contributed to heterogeneity in this group.

*Association of antidepressant use and oral HPV clearance*

In this study, recent use of antidepressants also appeared to be related to reduced oral HPV clearance though the effect was not as strong as that observed with use of antipsychotics. Evidence from *in vivo, in vitro,* and *ex vivo* studies indicate that antidepressants have anti-inflammatory effects including suppression of cell-mediated immunity and shifting of immune responses from a Th2-like to a Th1-like profile.[150-153] Accumulating evidence from clinical studies suggest antidepressants normalize elevated systemic cytokine levels,[203-206] although there are some conflicting results as well as limited data on long-term antidepressant use.[153,207] The immunomodulatory effects of antidepressants may explain why they are associated with reduced oral HPV clearance.

However, as with psychosis, it is possible that reduced clearance is associated with the immunomodulatory effects of depression itself, and not antidepressant use. Activation of the inflammatory response in depression has been well-documented, and depressive illness (including major depressive disorder and dysthymia, chronic low grade depression) is considered an inflammatory disease.[151,152,207-210] Additionally, infections and autoimmune disorders are strong risk factors for major depression so use of antidepressants may be a surrogate for other health factors that can influence oral HPV natural history.[211]

*Association of anxiolytics/sedatives and oral HPV clearance*

Similar to antipsychotics and antidepressants, benzodiazepines, a major class of anxiolytic/sedative medications, have immunomodulatory effects. *In vitro* and murine studies have found that benzodiazepines such as alprazolam, diazepam and midazolam can disrupt cytokine production as well as suppress macrophage activity and lymphocyte proliferation.[158-165] Studies in mice also indicate that benzodiazepines can increase susceptibility to infection, including pneumonia[161,212], *Salmonella thyphimurium*,[213] and orthopoxvirus infection.[214] These effects may be mediated through the interaction of benzodiazepines with peripheral $GABA_A$ receptors, which play an important role in immune responses at mucosal sites.[215] Most human data are from *ex vivo* and clinical studies of sedative use in patients in critical care settings, and administered by injection.[216] While these studies suggest sedatives have anti-inflammatory effects and may increase susceptibility to infection,[216] it is not conclusive whether these immunosuppressive effects are present when used as formulations intended for chronic

treatment (i.e. at lower doses and when taken orally). A large case-control study (n=34,661) found that use of commonly prescribed benzodiazepines, such as diazepam (i.e. Valium) and lorazepam (i.e. Ativan), were significantly associated with increased incidence of and mortality from community-acquired pneumonia,[217] which suggests immunosuppressive effects of benzodiazepines in outpatient usage. However, to our knowledge, no study has confirmed this via direct measurement of immune biomarkers in patients prescribed anxiolytic or sedative treatment for therapeutic indications. Therefore, while it is plausible that anxiolytic/sedative-induced immunomodulation may affect oral HPV clearance, this explanation is not conclusive. Also, previous research has focused on benzodiazepines, which are the most commonly prescribed anxiolytic/sedative. The immunomodulatory effects of other anxiolytic/sedative types are unknown.

The anxiolytics/sedatives user population is difficult to characterize, so while it is possible that illness-related characteristics may partially explain reduced clearance observed in users, this suggestion should be considered cautiously. Anxiolytics and sedatives are most often prescribed for generalized anxiety and stress, but they are used broadly for a number of other conditions including epileptic disorders, insomnia or even depressive symptoms. Neuropsychiatric disorders such as anxiety and stress can induce immunosuppression and alterations in peripheral cytokine profiles,[158,159] which may affect oral HPV clearance independent of anxiolytics/sedatives use. However, illnesses such as epilepsy or insomnia have no known direct immunomodulatory effects.

*Association of other prescription medications and oral HPV clearance*

In this study, erectile dysfunction medications were marginally associated with reduced clearance, but reasons for this finding are unclear. Literature on the immunomodulatory effects of these medications is sparse. One study showed that sildenafil (i.e. Viagra®) has immunomodulatory effects *in vivo* and *ex vivo* in healthy mice, and that these effects are immunosuppressive in male mice but immunostimulatory in female mice.[187] It is not known whether these same effects are present in humans, or if other erectile dysfunction medications besides sildenafil have similar side effects. The marginal association of erectile dysfunction medications with reduced clearance may be influenced by unmeasured sex-related risk factors that delay clearance in men, and that perhaps may be linked to the predominance of oral HPV in men, which has been consistently reported but not yet been fully explained.[1] Another potential explanation is that reduced clearance may be due to comorbidities associated with erectile dysfunction.[218] Sildenafil is becoming increasingly common as a treatment for pulmonary hypertension (Revatio®; sildenafil citrate), typically at a lower dose, so it may be helpful to explore differences in the effects of sildenafil by treatment indication.[219]

Other medications with known immunomodulatory effects, such as corticosteroids, were not associated with oral HPV clearance. This suggests that perhaps, underlying illness, or immune differences between individuals with certain illnesses, may be more predictive of oral HPV clearance than medication use.

*Effect of concomitant medication use on oral HPV clearance*

Our analysis of concomitant medication use suggests that neuropsychiatric comorbidities, or use of multiple medications to treat these disorders, may reduce oral HPV clearance. The effects of antipsychotics, antidepressants and anxiolytics/sedatives are difficult to disentangle especially since they are oftentimes prescribed together. Concurrent medication use may be due to comorbidity, severe or treatment-resistant illness, or multi-symptom clinical presentation.[201,202] For example, the clinical phenotypes for psychosis and depression overlap. Depression is common in patients with psychosis, with a prevalence of 25-75%. [220] Conversely, patients with depressive symptoms may exhibit symptoms of psychosis.[202] Sleep disturbances and insomnia are diagnosed in 30 to 80% of schizophrenic patients, [220] so concomitant use of anxiolytics/sedatives with antipsychotics is also not uncommon. The nuances of disease management may also result in multi-medication use. For example, a patient may experience several changes in medication types or dosages, or a trial period of concomitant medication usage, before finding a regimen that results in well-controlled depression.[202]

In our study population, 67% of the participants who reported using antipsychotics were also using antidepressants, which is comparable to the expected prevalence of clinical depression in patients with a psychosis disorder diagnosis. Simultaneous diagnoses of psychosis and depression appear to result in enhanced immunosuppression.[221] In a study of patients with first-episode psychosis, TNF-$\alpha$ and IL-4 were significantly higher in patients with a concurrent diagnosis of clinical depression, as compared to patients with psychosis only.[221] Therefore, the cumulative effects of

comorbidity, may explain why the oral HPV clearance is reduced to a greater degree with concurrent users of antipsychotics and antidepressants, as compared to users of only one or neither of these medications. While we examined the effects of recreational drug use and medication use on oral HPV infection separately, it is important to acknowledge that use of recreational drugs may influence medication clearance and activity levels, indirectly through effects on kidney and liver function, or directly through drug-medication interactions.[100,145]

Strengths of this study included the large population size, longitudinal follow-up and centralized testing of oral HPV infection. In addition, detailed time updated information on medication use was available and collected on validated survey instruments administered by experienced research staff that participants knew well.

This study had several limitations. Medication use was self-reported and we cannot exclude the possibility of misclassification bias. Data on dose and frequency of medication use were not available. Our analysis does not take into consideration differences in drug formulations or medication subtypes. In the future, research on newer drug types such as olanzapine for psychosis disorders, which is becoming more widely prescribed, may provide additional insight on the impact of these medications on oral HPV infection.

## Conclusion

In summary, we found significantly reduced oral HPV clearance in participants using antipsychotic medication, with potential differential effects by HIV status. Antidepressants and anxiolytics/sedatives may also be associated with reduced clearance,

especially when taken in combination. Each of these medications are prescribed for conditions that have immunomodulatory effects, so characteristics of the illness may partially contribute to reduced oral HPV clearance. The results of this study contribute evidence that immune differences may explain why some people clear oral HPV while others to do not.


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**Table 5-1.** Participant characteristics at time of first oral HPV detection

| Participant characteristic[1] | No. | % |
|---|---|---|
| **Sex** | | |
| Women (WIHS) | 274 | 46% |
| Men (MACS) | 320 | 54% |
| **Infection type[2]** | | |
| Incident only | 189 | 32% |
| Any Prevalent | 405 | 68% |
| **Age (year)** | | |
| <45 | 162 | 27% |
| 45-54 | 258 | 43% |
| ≥55 | 174 | 29% |
| **Race/Ethnicity** | | |
| White, non-Hispanic | 186 | 31% |
| Black, non-Hispanic | 328 | 55% |
| Other race or Hispanic | 80 | 14% |
| **Study site[3]** | | |
| Baltimore, Maryland | 88 | 15% |
| Bronx, New York | 93 | 16% |
| Brooklyn, New York | 84 | 14% |
| Chicago, Illinois | 202 | 34% |
| Los Angeles, California | 24 | 4% |
| Pittsburgh, Pennsylvania | 103 | 17% |
| **HIV status** | | |
| Uninfected | 168 | 28% |
| Infected | 426 | 72% |
| **Current CD4 T cell count (cells/uL)** | | |
| ≥500 | 212 | 50% |
| <500 | 214 | 50% |
| **Current HIV RNA viral load (copies/mL)** | | |
| <200 | 286 | 68% |
| 200-19,999 | 78 | 19% |
| ≥20,000 | 57 | 14% |
| **Current ART use** | | |
| No | 83 | 20% |
| Yes | 343 | 81% |
| **Amount of saliva (self-reported)** | | |
| Don't notice/normal | 421 | 77% |
| Too little/dry mouth | 82 | 15% |
| Too much | 46 | 8% |

[1] All participant characteristics are from the time of oral HPV detection, with the exception of amount of saliva, which was reported at time of POPS enrollment.
[2] Prevalent infection was defined as oral HPV detected at POPS enrollment. Incident infection was defined as newly detected oral HPV preceded by at least one HPV-negative oral rinse. Some participants had prevalent infection, followed by incident infection of another HPV type that was included in the analysis.
[3] Baltimore, Los Angeles and Pittsburgh study sites enrolled men only (MACS). Bronx and Brooklyn study sites enrolled women only (WIHS). Chicago study site enrolled both men and women.

**Table 5-2.** Unadjusted associations of recent medication use with time to oral HPV clearance among 594 participants with 1,358 oral HPV infections

| Medication type used since last visit[1] | No. of cleared infections | Person-months of observation | HR (95% CI) |
|---|---|---|---|
| **Antipsychotics[2]** | | | |
| **HIV-uninfected (n=299)** | | | |
| No | 122 | 3,968 | Ref |
| Yes | 13 | 273 | 1.38 (0.88-2.17) |
| **HIV-infected (n=1,059)** | | | |
| No | 466 | 14,998 | Ref |
| Yes | 46 | 2,298 | **0.67 (0.49-0.91)** |
| **Anxiolytics/sedatives** | | | |
| No | 512 | 16,123 | Ref |
| Yes | 135 | 5,415 | **0.78 (0.63-0.96)** |
| **Antidepressants** | | | |
| No | 466 | 14,415 | Ref |
| Yes | 181 | 7,123 | **0.82 (0.67-0.999)** |
| **Asthma (steroidal)** | | | |
| No | 572 | 19,288 | Ref |
| Yes | 75 | 2,250 | 1.20 (0.89-1.63) |
| **Asthma (non-steroidal)[3]** | | | |
| No | 489 | 16,349 | Ref |
| Yes | 158 | 5,189 | 1.06 (0.87-1.28) |
| **Cholesterol** | | | |
| No | 469 | 15,742 | Ref |
| Yes | 178 | 5,796 | 1.03 (0.86-1.25) |
| **Diabetes** | | | |
| No | 567 | 19,231 | Ref |
| Yes | 80 | 2,307 | 1.19 (0.92-1.53) |
| **Hormones** | | | |
| No | 618 | 20,665 | Ref |
| Yes | 29 | 872 | 1.10 (0.72-1.69) |
| **Hypertension** | | | |
| No | 397 | 13,246 | Ref |
| Yes | 250 | 8,292 | 1.02 (0.86-1.22) |
| **NSAIDs** | | | |
| No | 419 | 11,760 | Ref |
| Yes | 203 | 6,673 | 0.90 (0.76-1.07) |
| **Erectile dysfunction (men only)** | | | |
| No | 296 | 9,952 | Ref |

| | | | |
|---|---|---|---|
| Yes | 56 | 2,419 | 0.77 (0.58-1.01) |

[1] All medication use variables were time-updated (i.e. medication use status reported at each visit during oral HPV infection was applied to the WLW model, and was allowed to change if the participant started/stopped medication use). If a participant missed a visit, the last reported medication use status was used in the analysis.
Statistically significant values are listed in bold. Statistical significance was defined as two-sided p-value <0.05 or 95% confidence interval that does not include 1.0.
[2] Antipsychotic medication use was associated with reduced clearance in HIV-infected participants only (p-interaction=0.009)
[3] There appeared to be effect modification by HIV status for non-steroidal antiasthmatics (p-interaction=0.012), although the association with reduced clearance in HIV-infected participants was non-significant (p=0.73).

**Table 5-3.** Multivariable risk factors for oral HPV clearance among 594 participants with 1,358 oral HPV infections[1]

| Participant characteristic[2] | aHR (95% CI) |
|---|---|
| **Antipsychotics use**[3] | |
| **HIV-uninfected** | |
| No | Ref |
| Yes | 1.62 (0.96-2.73) |
| **HIV-infected** | |
| No | Ref |
| Yes | **0.66 (0.48-0.90)** |
| **Anxiolytics/sedatives use** | |
| No | Ref |
| Yes | 0.89 (0.73-1.07) |
| **Antidepressant use** | |
| No | Ref |
| Yes | 0.89 (0.71-1.11) |
| **Sex** | |
| Women (WIHS) | Ref |
| Men (MACS) | 0.87 (0.73-1.04) |
| **Infection type** | |
| Incident | Ref |
| Prevalent | **0.47 (0.39-0.56)** |
| **Age** | |
| <45 | Ref |
| 45-54 | 0.91 (0.75-1.11) |
| ≥55 | **0.79 (0.56-0.97)** |
| | p-trend=0.05 |
| **HIV status and CD4 T cell count** | |
| HIV negative | Ref |
| HIV positive, CD4≥500 | 0.91 (0.72-1.15) |
| HIV positive, CD4<500 | 0.84 (0.67-1.04) |
| | p-trend=0.11 |

[1] Covariates shown are for multivariable model with antipsychotics. Similar covariates for model with antidepressants and model with tranquilizers. Each medication modeled separately.

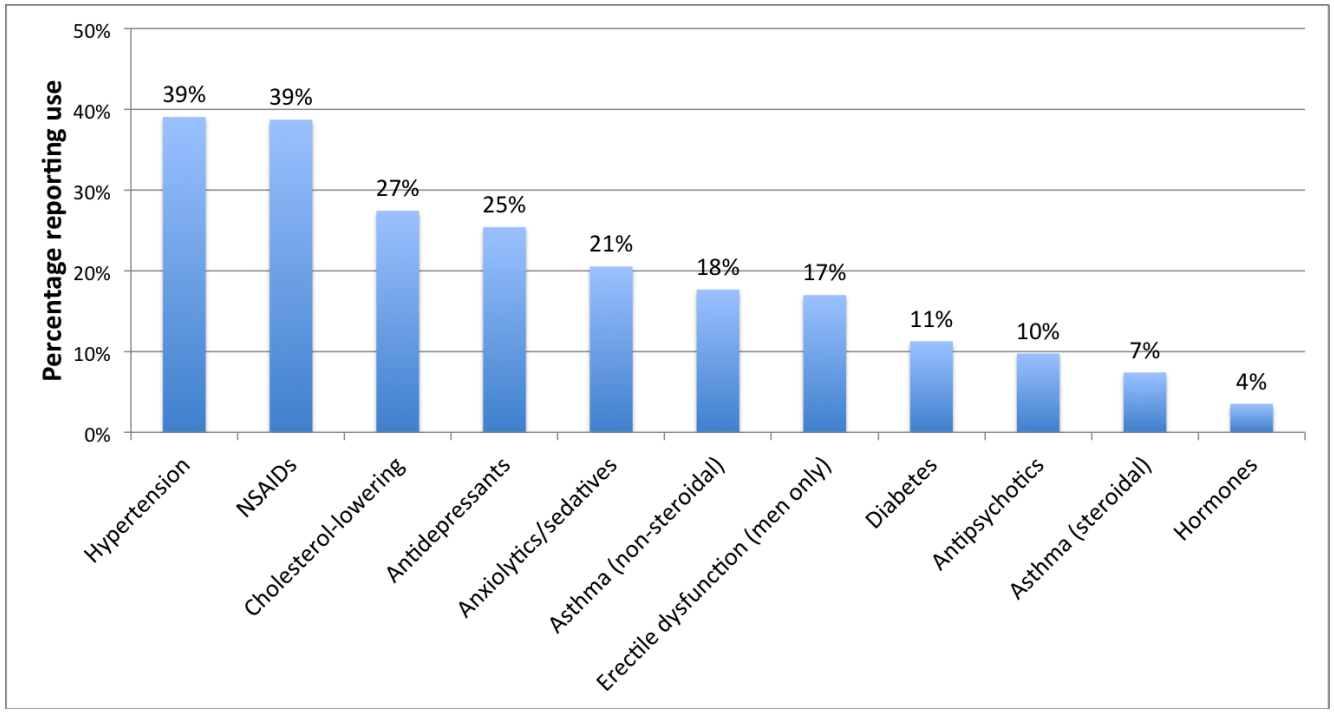
[2] All medication use variables were time-updated and refer to use of medication since the last study visit. HIV status and CD4 count were not time-updated; value from visit of oral HPV detection was used in the analysis

Statistically significant values are listed in bold. Statistical significance was defined as two-sided p-value <0.05 or 95% confidence interval that does not include 1.0.

Adjusted for cohort/sex, type of infection (prevalent/incident), age, HIV status and CD4 count; aHR = adjusted hazard ratio

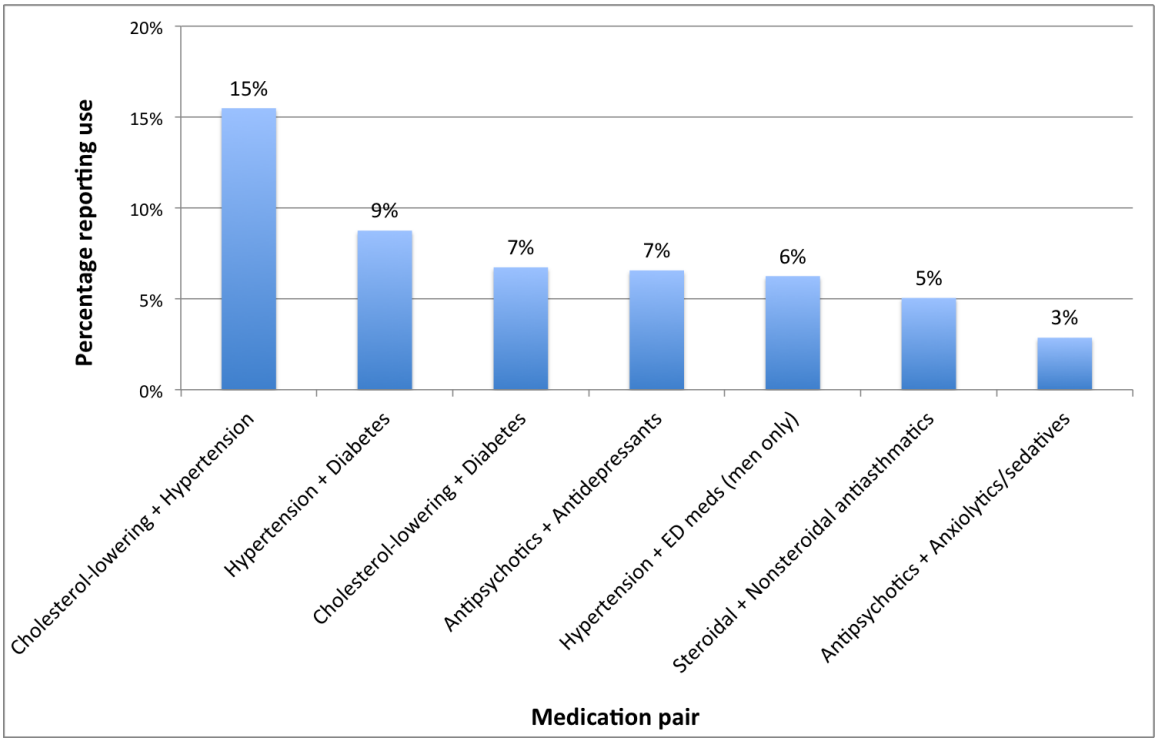
[3] Antipsychotic medication use was associated with reduced clearance in HIV-infected participants only (p-interaction=0.009)

**Figure 5-1.** Prevalence of medication use at time of first oral HPV detection



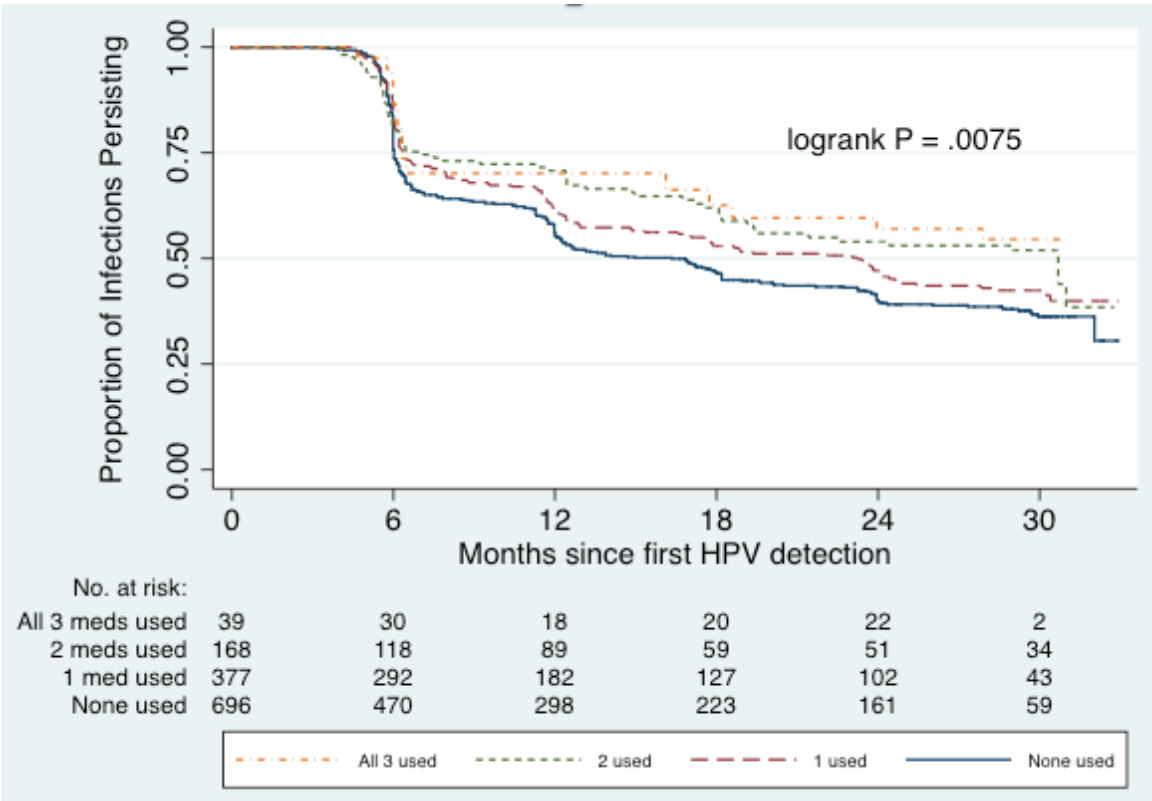

Medication use is shown for men (n=320) and women (n=274) together, with the exception of erectile dysfunction medications, which were assessed in men only.
NSAID = non-steroidal anti-inflammatory drugs

**Figure 5-2.** Prevalence of concomitant medication use at time of oral HPV detection




Medication use is shown for men (n=320) and women (n=274) together, with the exception of erectile dysfunction medications, which were assessed in men only.

**Figure 5-3.** Time to oral HPV clearance among 594 participants with 1,358 oral HPV infections, by use of antipsychotics, antidepressants and/or anxiolytics/sedatives



# Chapter 6. Epidemiology of HPV-associated cancers in Singapore

This chapter is modified from a paper published in PLOS ONE. "Incidence, trends and ethnic differences of oropharyngeal, anal and cervical cancers: Singapore, 1968-2012"[222]


## Abstract

**Background:** In recent decades, several Western countries have reported an increase in oropharyngeal and anal cancers caused by human papillomavirus (HPV). Trends in HPV-associated cancers in Asia have not been as well described.

**Methods:** We describe the epidemiology of potentially HPV-related cancers reported to the Singapore Cancer Registry from 1968-2012. Analysis included 998 oropharyngeal squamous cell carcinoma (OPSCC), 183 anal squamous cell carcinoma (ASCC) and 8,019 invasive cervical cancer (ICC) cases. Additionally, 368 anal non-squamous cell carcinoma (ANSCC), 2,018 non-oropharyngeal head and neck carcinoma (non-OP HNC), and 11,627 nasopharyngeal cancer (NPC) cases were included as comparators. Age-standardized incidence rates (ASR) were determined by gender and ethnicity (Chinese, Malay and Indian). Joinpoint regression was used to evaluate annual percentage change (APC) in incidence.

**Results:** OPSCC incidence increased in both genders (men 1993-2012, APC=1.9%, $p<0.001$; women 1968-2012, APC=2.0%, $p=0.01$) and was 5 times higher in men than women. In contrast, non-OP HNC incidence declined between 1968-2012 among men

(APC= -1.6%, p<0.001) and women (APC= -0.4%, p=0.06). NPC incidence also declined in both genders (men 1988-2012, APC= -2.64%, p<0.001; women 1988-2012, APC= -3.92%, p<0.001). ASCC and ANSCC were rare (ASR=0.2 and 0.7 per 100,000 person-years, respectively) and did not change significantly over time except for increasing ANSCCs in men (APC=2.8%, p<0.001). ICC was the most common HPV-associated cancer (ASR=19.9 per 100,000 person-years) but declined significantly between 1968-2012 (APC= -2.4%). Incidence of each cancer varied across ethnicities.

**Conclusions:** Similar to trends in Western countries, OPSCC incidence increased in recent years, while non-OP HNC decreased. In contrast to OPSCC, NPC incidence also decreased in recent years, suggesting a shift in risk factor profile for head and neck cancers in Singapore. ICC remains the most common HPV-related cancer in Singapore, but Pap screening programs have led to consistently decreasing incidence.

## Background and rationale

Human papillomavirus (HPV), a common sexually transmitted infection, causes approximately 4.8% of all cancers worldwide, including nearly 100% of cervical cancers, most (90%) anal cancers, and 35-80% of oropharyngeal cancers.[6,8,10,223,224] Epidemiologic and molecular data indicate that HPV is an increasingly important risk factor for cancer, especially as tobacco-related cancers decline in many countries.[2,10,20,225]

The epidemiology of HPV-associated cancers varies geographically and between racial/ethnic groups, likely due to differences in patterns of tobacco use or in sexual behaviors that lead to HPV infection.[10,17,20,223,226-231] Accumulating evidence from countries in North America and Europe (e.g. USA, Canada, Denmark, The Netherlands, the UK, Norway) show that the incidence of HPV-associated oropharyngeal squamous cell carcinoma (OPSCC) and anal squamous cell carcinoma (ASCC) has increased over the past 2 to 4 decades, particularly among men.[2,18,225-234] Data on the epidemiology of HPV-associated cancers in Asia are more limited, but cancer registry-based studies in Korea and Taiwan found similar increases in HPV-related OPSCC.[235,236] Other studies suggest that the incidence of OPSCC in some regions (e.g. Hong Kong, Chennai India, Singapore) was stable or decreasing from the 1980s to early 2000s.[17,20] Trends across Asian ethnic groups have not been previously studied. Ethnic disparities have been reported in Singapore and other parts of Asia for some infection-associated cancers including cervical,[237,238] nasopharyngeal[237-239] and liver cancers,[237,238] but are less clear for oropharyngeal and anal cancers.

To contribute Asian data on this topic, we sought to characterize the epidemiology of potentially HPV-associated cancers in Singapore, focusing on

oropharyngeal, anal and cervical cancers. The epidemiology of nasopharyngeal cancers was also evaluated, as it is an infection-associated head and neck cancer endemic to this region and is known to affect Asian ethnic subgroups disproportionately.[239] Singapore is a high-income, multicultural city-state of 4 million residents in Southeast Asia with 3 primary ethnicities – Chinese (74.2%), Malay (13.3%) and Indian (9.2%), which allowed description of ethnic variations in cancer incidence.[240] We evaluated oropharyngeal, anal, cervical and nasopharyngeal cancers in Singapore over 4 decades to: 1) determine cancer incidence, by type, gender and ethnicity, and 2) characterize temporal trends in these cancers.

## Methods

*Case inclusion and classification*

We restricted our analysis to confirmed incident cancers at anatomic sites where HPV is known to be a primary cause, including oropharyngeal squamous cell carcinoma (OPSCC), anal squamous cell carcinoma (ASCC) and invasive cervical cancer (ICC). We included non-oropharyngeal head and neck squamous cell carcinoma (non-OP HNC), which is primarily tobacco-related,[1] and nasopharyngeal cancer (NPC), which is Epstein-Barr virus (EBV)-related,[241] as comparators to OPSCC. We included invasive anal non-squamous cell carcinoma (ANSCC) as a comparator to ASCC.

Cancer sites were defined according to International Classification of Diseases codes for oncology (ICD-O-3). Tumor HPV status was not available, so tumor site was used as a proxy to classify cases as "HPV-related" and "HPV-unrelated", similar to previous research.[18,20,225,226,230,232,233,235,236,242] OPSCC sites included the oropharynx (C10.0-C10.4, C10.8-C10.9), tonsil (C02.4, C09.0-C09.1, C09.8-C09.9), base of tongue

(C01.9), soft palate and uvula (C05.1-C05.2), and Waldeyer's ring (C14.2). Non-OP HNC sites included other parts of the tongue (C02.0-C02.3, C02.8-C02.9), mouth (C04.0-C04.1, C04.8-C04.9; C06.0-C06.2, C06.8-C06.9), gum (C03.0-C03.1, C03.9), and hard palate (C05.0, C05.8-C05.9). OPSCC and non-OP HNC analyses were restricted to cancers with squamous cell histologies (ICD-O-3 codes: 8050 to 8076, 8078, 8083, 8084, 8094). ICC included endocervix (C53.0), exocervix (C53.1), and cervix uteri (C53.8, C53.9). All histologic types of ICC were included as nearly all ICC is due to HPV infection.[224] Invasive anal cancers (C21.0-C21.8) were subdivided into squamous and non-squamous histology. Historically, there has not been a widely accepted definition of the outer limits of the anal canal.[228] To reduce likelihood of misclassification error, we excluded perianal cancers, which were coded such that they could include skin cancers. It is possible that some more distally located HPV-associated anal cancers may be coded as skin or perianal cancers, so our classification of potentially HPV-associated anal cancers is conservative. NPC included all nasopharynx sites (C11.0-C11.3, C11.8-C11.9).

*Data sources*

The numbers of newly diagnosed cancer cases reported between 1968-2012 were obtained from the Singapore Cancer Registry, a population-based registry covering all Singapore residents. The Ministry of Health Singapore enacted the National Registry of Diseases Act in 2007 to ensure comprehensive notifications of cancer cases (local and foreign residents) by healthcare institutions in Singapore. The Singapore Cancer Registry includes 1.09% death certificate only cases and 91.8% morphologically verified cases (unpublished information). Case counts were obtained in aggregate form, by 5-year

calendar period (i.e. 1968-72, 1973-77…2008-2012) and age groups (i.e. 20-24, 25-29…65-69, ≥70). Data were further subdivided by gender and ethnicity. Cancer registry information was based on data from medical professionals, pathology records and hospital records.[238] Population denominators for incidence rates were derived from mid-year population estimates from the Singapore Department of Statistics for each year from 1968 to 2010 and extrapolated for 2011 and 2012 based on 2010 estimates. Prior to 1980, population denominator data collected by the Singapore Department of Statistics included individuals on temporary residence permits (e.g. work permits, student passes) while cancer case counts included residents only. This inflation of the denominator could have resulted in an underestimation of incidence prior to 1980; however, the non-resident population was small during this time so underestimation is expected to be minimal. Our analysis used aggregated, de-identified patient data only. Permission was obtained from the Singapore National Diseases Registry Office and was approved as exempt from IRB review by the National University of Singapore IRB.

*Statistical analyses*

Crude incidence rates for each 5-year period were calculated for each cancer type, overall and by gender and ethnicity. Age-standardized incidence rates (ASR) per 100,000 person-years were calculated using the direct method[243] and based on the WHO world standard population.[244] Incidence rate ratios (IRRs) compared men and women overall and for each cancer type. Stata 12 software was used.[73]

Temporal trends in ASR (5-year periods) for each cancer were characterized using the Joinpoint Regression Program, version 4.1.1.[245] This method uses least squares

regression to fit line segments to the natural log of the ASR, joined at discrete points (midpoint of 5-year periods) identified by the software to represent statistically significant changes in direction of trend.[245] Models with a minimum of 0 joinpoints (1 straight line) and maximum of 1 joinpoint (two line segments) were tested sequentially using Monte Carlo permutation tests to determine the appropriate number and placement of joinpoints, if any.[246] The simpler, straight-line model was chosen as the final model unless the addition of a joinpoint resulted in a significantly better fit. The average annual percentage change (APC) in ASR was calculated and considered significant at $p \leq 0.05$. Temporal trends were explored by gender for all cancer types. Temporal trends in ICC and NPC were also explored when stratified by Chinese, Malay and Indian ethnicities. The "Other ethnicity" category was excluded from ethnicity-stratified analyses because the number of cancers was too few for reliable results, but was included in overall and gender-stratified analyses. Due to low numbers, ethnicity-stratified joinpoint results are not reported for OPSCC, non-OP HNC and anal cancers.

## Results

Between 1968-2012, 9,200 potentially HPV-associated cancers were diagnosed in Singapore including 998 OPSCC, 183 ASCC and 8,019 ICC. There were 2,018 non-OPC HNC 368 ANSCC and 11,627 NPC diagnosed during the same period (Table 6-1). The incidence of each cancer increased with age, and the median age at diagnosis for OPSCC, non-OP HNC, ASCC, ANSCC, ICC and NPC was 62, 61, 66, 66, 53 and 48 years, respectively.

Gender disparities in age-standardized incidence rates were observed for most cancer types. OPSCC, non-OPC HNC, ANSCC and NPC occurred significantly more frequently in men than women, while ASCC incidence was similar in men and women (Table 6-2). ICC accounted for 87% of all HPV-associated cancers (ASR=19.9 per 100,000 person-years). The incidence of OPSCC (ASR=1.4 per 100,000 person-years) and ASCC (ASR=0.3 per 100,000 person-years) were lower.

*Incidence trends*

Trends in OPSCC and non-OP HNC (Figure 6-1), ASCC and ANSCC (Figure 6-2), ICC (Figure 6-3) and NPC (Figure 6-4) between 1968-2012 were explored. In the last 20 years (1993-2012), OPSCC incidence increased steadily in men (APC=1.9%, $p<0.001$) and women (APC=2.0%, $p=0.01$) (Figure 6-1). In contrast, non-OP HNC incidence decreased in men (APC=-1.6, $p<0.001$) and women (APC=-0.4, $p=0.06$) during this time period. In previous time periods (1968-1992), the incidence of both OPSCC and non-OP HNC decreased in men, but only decreased for non-OP HNC in women (Figure 6-1).

Analyses of anal carcinomas by histologic subtype suggested different trends over time in ASCC and ANSCC. ASCC incidence appeared to decrease from 1968 to 2012 in both genders. In contrast, ANSCC incidence appeared to increase during this same period (Figure 6-2). The increase in ANSCC incidence in men was significant (APC=2.8%, $p<0.001$), but other observed ASCC and ANSCC trends were not statistically significant.

ICC incidence declined consistently across all ethnicities, most notably in Indian women, who experienced an average -6.78% ($p<0.001$) decrease in ASR per year

between 1978-2012; ICC incidence decreased from 47.2 to 4.7 per 100,000 person-years during this time period (Figure 6-3, Table 6-4). ICC incidence also decreased in Chinese women, primarily from 1993-2012 (APC= -4.8%, $p<0.001$). In the most recent time period (2008-2012), the overall incidence of ICC in Singapore (ASR=11.6 per 100,000 person-years) remained lower than the global estimated burden (ASR=15.2 per 100,000 person-years) but slightly higher than the average rate in more developed countries (ASR=9.0 per 100,000 person-years).[247]

NPC incidence declined consistently for Chinese men and women, with the greatest drop in incidence occurring between 1988-2012 (APC, men= -2.64%, $p<0.001$; APC, women= -3.92%, $p<0.001$) (Figure 6-4). Rates in Malay men increased during this same period (APC=1.10, $p=0.01$) while rates in Malay women, Indian men and Indian women remained consistently low and stable from 1968-2012 (Figure 6-4). In the most recent time period (2008-2012), the overall NPC incidence in Singapore (ASR=9.7 per 100,000 person-years) remains higher than NPC incidence in parts of the world where this cancer is not endemic (<1 per 100,000 person-years).

*Ethnic differences*

Ethnic differences in the incidence of potentially HPV-associated cancers (OPC, ASCC and ICC) were observed. Chinese women had higher risk of HPV-associated cancer overall (ASR=22.0 per 100,000 person-years) compared to Malay (ASR=14.7, $p<0.001$) or Indian (ASR=14.9, $p<0.001$) women, primarily due to higher ICC incidence in Chinese women (Table 6-3). In contrast, Indian men had higher risk of HPV-associated cancer (ASR=4.0 per 100,000 person-years) compared to Chinese (ASR=2.7,

p<0.001) or Malay (ASR=1.1, p<0.001) men, primarily due to high OPSCC incidence among Indian men (Table 6-3; Table 6-5). When considering only the most recent time period (2008-2012), these same ethnic variations remain.

## Discussion

This study suggests that there are gender and ethnic differences in the incidence and temporal trends of potentially HPV-associated cancers in Singapore. Incidence of HPV-associated cancer overall was higher in women than men, due to the burden of cervical cancer among women, but OPSCC incidence was significantly higher in men than women. Ethnic differences in incidence of HPV-associated cancer were observed, with higher rates overall among Indian men and among Chinese women than other ethnicities. Over the 45 years studied, ICC, non-OP HNC and NPC incidence decreased significantly in Singapore, but OPSCC rates increased in both men and women in recent years. This research suggests the distribution of HPV-associated cancers across population subgroups has changed over the past few decades, possibly mirroring changes in tobacco and sexual risk factors.

Similar to trends reported in other countries of comparable socioeconomic status, the incidence of OPSCC in Singapore is rising.[2,10,17,18,20,225,226,230-236] However, unlike some other countries where this increase was only observed in men,[1,2,10] OPSCC incidence in Singapore appeared to increase for both genders. Recent research suggests increases in OPSCC are largely explained by HPV and are likely driven by changing sexual practices.[10,20,231,248] In the current study, tumor HPV status was not available, so we do not know what proportion of the OPSCC cases included in our analysis was HPV-

positive and how this proportion differed by gender and ethnicity, or changed over time. Given that the observed patterns in OPSCC incidence differed in men and women, it is possible that changing sexual practices may not fully explain trends.

The recent increasing trend in OPSCC contrasts with consistently decreasing rates of non-OP HNC, which are more strongly tied to tobacco usage. Decreasing OPSCC incidence in men in the 1970s and 1980s is likely related to declining tobacco use, including decreasing popularity of traditional smoking methods such as hand-rolled cigarettes ("ang hoon"). Smoking prevalence in Singapore has been declining in men since the 1970s and has remained low (<5%) in women,[249,250] consistent with observed decreases in non-OP HNC in this study and decreasing lung cancer rates reported elsewhere.[238,250] The overall incidence of non-OP HNC in Singapore is lower than that in North America and Europe,[17,251] consistent with the lower smoking rate in Singapore (~13%).[249] Tobacco use in Singapore is amongst the lowest in developed countries, largely due to the success of anti-tobacco campaigns, legislation on tobacco taxation and prohibition of smoking in public places.[249] Given the low smoking prevalence, the epidemiology of non-OPC in Singapore may reflect the HNC profile we will see in other settings as tobacco cessation efforts continue.

Increasing OPSCC also contrasts with decreasing NPC, which is a non-HPV-related head and neck cancer. Similar decreases in NPC incidence have been observed in other urban EBV-endemic regions with large Chinese populations, such as Hong Kong, Shanghai and Taiwan,[252-255] and have been attributed to diet and lifestyle changes, particularly since NPC incidence has remained stable in rural areas or parts of Mainland China where traditional dietary practices have been maintained.[239,256,257] Higher NPC risk

among ethnic Chinese is well-known, and this is reflected in several-fold lower incidence in Malay and Indian ethnicities across all time periods studied.[239] Increasing NPC incidence among Malay men has been previously reported and may reflect changes in environmental risk factors, including occupational exposures in the migrant Malay population.[258] Although NPC remains the most common cause of head and neck cancer in Singapore, declining trends are encouraging. Furthermore, the divergent OPSCC and NPC trends highlight the distinct risk factor profiles of these cancers; increasing OPSCC may be an emerging health concern especially if overall NPC incidence continues to decline.

Overall, anal cancer is an uncommon malignancy in Singapore. The observed ASCC and ANSCC rates were 2- to 4-fold lower in Singapore than in Western countries (typically 1-2 cases per 100,000 in the general population), probably reflecting differences in sexual habits in the Singapore population.[228] Unlike some other countries, ANSCC in Singapore is more common among men than women, although numbers for both genders are low.[259,260] The observed increase in incidence of ANSCC is similar to the increasing incidence of colorectal cancer during roughly this same time period.[261] Colorectal cancer is the most common cancer among males in Singapore, primarily has non-squamous cell histology and increases in incidence have been attributed to diet and lifestyle changes in older men.[261] If ANSCC and colorectal cancer have similar risk factors, this could explain the sharp increase in ANSCC observed during the study period. The strongly divergent trends in ASCC and ANSCC over time suggest etiologic differences and the importance of distinguishing anal cancers by histologic type in future reports of cancers at HPV-related subsites.

Despite substantial decreases in the incidence of ICC in Singapore over the past 4 decades, ICC remains the most common HPV-associated cancer in Singapore. The incidence of ICC in Singapore remains high compared to Western countries of similar economic status.[247,262] However, consistent with previous studies,[238,262] we observed an encouraging decline in ICC rates during the time period of our study. This is largely attributed to opportunistic Pap screening which has been available since 1964, and a national cervical cancer screening program implemented in 2004, both of which have contributed to early detection and treatment of cervical pre-cancers.[262-264]

The national ICC screening program, which targets sexually active women starting at age 25, has successfully expanded coverage with comparable reach for Chinese, Malay and Indian ethnicities for first screens.[262,265] Health surveys conducted in 2008 and 2010 found that Malay women had a higher rate of loss to re-screen, followed by Indian and then Chinese women, which may explain why ICC rates have decreased the least in Malay women.[263,265] However, it is unclear why Chinese women remain at higher risk of ICC, as compared to Malay and Indian women in Singapore. In the future, increasing Pap screening coverage above the current 50-80%, and ensuring women receive rescreens within the recommended time interval, may reduce ICC rates further.[263] HPV vaccines (Gardasil®, Merck & Co., Inc., Whitehouse Station, NJ, USA and Cervarix®, GlaxoSmithKline Biologicals, Rixensart, Belgium) are licensed for use in Singapore, but must be covered by out-of-pocket expenses, or Medisave, a compulsory health savings scheme where individuals put aside part of their income to pay future medical expenses for themselves or dependents.[262,266] Implementation of a national HPV

vaccination program or provision of subsidies for low-income individuals could also contribute to future declines in incidence of ICC, and other HPV-associated cancers.

Although women experience the greater HPV-associated cancer burden in Singapore, our results suggest that men may also benefit from prevention efforts targeting these cancers as they bear a greater risk of OPSCC. Compared to other cancers in Singapore,[238] OPSCC is relatively rare, and rates in Singapore are lower than in other economically developed countries.[2,17] However, men are disproportionately affected and rates are increasing. Although HPV vaccination efforts in Singapore are primarily focused on women, and the impact of HPV vaccine on OPSCC has not been fully studied, initial research suggests HPV vaccination will likely also be effective in preventing oral HPV infection and thus could impact OPSCC rates.[267,268] Should HPV prevention efforts be scaled up in Singapore, our study provides baseline data on the incidence of potentially HPV-associated cancers during time periods when HPV vaccination coverage is minimal.

Although the cancers in our analysis are at HPV-related sites, HPV may be just one of the factors contributing to observed cancer epidemiology. The heterogeneity in patterns of HPV-associated cancer that we observed across ethnic groups could reflect the effects of sociocultural practices, genetics, environmental exposures or an interaction between etiologic factors. Since the 1960s, Singapore has undergone rapid economic development and has become increasingly 'westernized' in its transition from a developing country to a high-income country and commercial hub in Southeast Asia. This has been accompanied by changes in diet, lifestyle and customs that have been cited

as contributing factors to the increasing burden of chronic infections and cancer.[261,262,266,269,270]

Differences between ethnic groups in lifestyle factors such as tobacco use and sexual norms may contribute to observed differences in HPV-related cancer incidence. For instance, Indians are known to have a high prevalence of tobacco and betel quid use, practices which increase risk of head and neck cancers.[271-274] The higher incidence of OPSCC observed in Indian Singaporean men and women could be due to early life exposure to betel quid in their country of origin or continuation of betel use habits after immigration to Singapore. Lower incidence of non-OP HNC among Malays is surprising given that their smoking rates are higher than the general Singaporean population (18.6% to 30.8% between 1979-2010),[250] but Malays also have low lung cancer incidence suggesting that factors other than smoking exposure may account for differences in cancer incidence.[250,273] Overall, Malays have the lowest HPV-associated cancer incidence, compared to Chinese and Indian ethnic groups. This may be due to lower risk of HPV infection, or possibly other lifestyle factors that may be protective of cancers. Most Malays are Muslim, and they may adhere to a more traditional lifestyle (i.e. diet, sexual behaviors) despite Singapore's modern environment.[258] It is also possible that ethnicity does not fully encompass behavioral, sociocultural and genetic differences and that the broad ethnic categorizations used may obscure relevant within-group differences in practices or behaviors.

Limitations of this study include low numbers of OPSCC and ASCC, and lack of data on tumor HPV status and behavioral risk factors, including smoking and betel quid use. Additionally, some cases had insufficient information to identify a precise tumor site

and were classified as ICD-O-3 NOS ("not otherwise specified" tumor site) in the Singapore Cancer Registry; thus, misclassification of these cases is another potential limitation. Strengths of this study include the use of high-quality cancer registry data that is representative of the Singaporean resident population, the long time-interval (>40 years) included in the analysis, and the Asian ethnic variation explored.

## Conclusions

Our study provides a snapshot of the current burden and recent trends of oropharyngeal, anal, cervical and nasopharyngeal cancers in Singapore, a multi-ethnic setting where HPV vaccination is not yet widespread. Although HPV-associated cancer prevention in Singapore has primarily focused on cervical cancer, our study shows for the first time that there is also a burden of potentially HPV-related OPSCC in men, and that the incidence is rising, in contrast to non-HPV-related OPSCC and nasopharyngeal cancers. Furthermore, our study illustrates differences in burden of these cancers by Asian ethnicities, underscoring the need to understand differences in risk factors across population subgroups in Singapore's diverse setting. With progressively increasing industrialization and population growth, the epidemiology of these cancers in Singapore may reflect the cancer profile we will see in other settings as tobacco cessation efforts continue. Understanding the changing epidemiology of HPV-associated cancers is important for cancer prevention and provides a picture of cancer risk in a population with low tobacco use.

## Acknowledgements

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**Table 6-1.** Trends in crude cancer incidence over time, by type and gender, from 1968 to 2012 in Singapore[1]

| Gender | Year of diagnosis | Person-years | Oropharyngeal | | Non-oropharyngeal HNC | | Anal SCC | | Anal non-SCC | | Invasive cervical cancer[2] | | Nasopharyngeal | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | n | cIR | n | IR | n | cIR | n | cIR | n | cIR | n | cIR |
| **Men** | **1968-1972** | 2,608,200 | 59 | 2.26 | 93 | 3.57 | 4 | 0.15 | 5 | 0.19 | NA | NA | 547 | 20.97 |
| | **1973-1977** | 3,111,100 | 59 | 1.90 | 110 | 3.54 | 9 | 0.29 | 8 | 0.26 | NA | NA | 665 | 21.38 |
| | **1978-1982** | 3,576,850 | 55 | 1.54 | 123 | 3.44 | 4 | 0.11 | 7 | 0.20 | NA | NA | 689 | 19.26 |
| | **1983-1987** | 4,108,400 | 64 | 1.56 | 145 | 3.53 | 7 | 0.17 | 17 | 0.41 | NA | NA | 861 | 20.96 |
| | **1988-1992** | 4,700,300 | 74 | 1.57 | 135 | 2.87 | 8 | 0.17 | 23 | 0.49 | NA | NA | 1,059 | 22.53 |
| | **1993-1997** | 5,276,530 | 76 | 1.44 | 156 | 2.96 | 6 | 0.11 | 18 | 0.34 | NA | NA | 1,135 | 21.51 |
| | **1998-2002** | 5,784,960 | 119 | 2.06 | 194 | 3.35 | 9 | 0.16 | 25 | 0.43 | NA | NA | 1,108 | 19.15 |
| | **2003-2007** | 6,216,165 | 139 | 2.24 | 181 | 2.91 | 11 | 0.18 | 49 | 0.79 | NA | NA | 1,213 | 19.51 |
| | **2008-2012** | 6,871,900 | 183 | 2.66 | 213 | 3.10 | 19 | 0.28 | 69 | 1.00 | NA | NA | 1,157 | 16.84 |
| | **TOTAL** | **42,254,405** | **828** | **1.96** | **1,350** | **3.19** | **77** | **0.18** | **221** | **0.52** | **NA** | **NA** | **8,434** | **19.96** |
| **Women** | **1968-1972** | 2,477,300 | 4 | 0.16 | 39 | 1.57 | 4 | 0.16 | 4 | 0.16 | 603 | 24.34 | 216 | 8.72 |
| | **1973-1977** | 3,020,100 | 4 | 0.13 | 34 | 1.13 | 5 | 0.17 | 7 | 0.23 | 676 | 22.38 | 266 | 8.81 |
| | **1978-1982** | 3,519,250 | 15 | 0.43 | 53 | 1.51 | 12 | 0.34 | 2 | 0.06 | 751 | 21.34 | 322 | 9.15 |
| | **1983-1987** | 4,079,100 | 10 | 0.25 | 56 | 1.37 | 10 | 0.25 | 11 | 0.27 | 896 | 21.97 | 371 | 9.10 |
| | **1988-1992** | 4,682,260 | 14 | 0.30 | 68 | 1.45 | 9 | 0.19 | 11 | 0.23 | 998 | 21.31 | 440 | 9.40 |
| | **1993-1997** | 5,349,900 | 15 | 0.28 | 84 | 1.57 | 6 | 0.11 | 25 | 0.47 | 1,130 | 21.12 | 414 | 7.74 |
| | **1998-2002** | 5,960,376 | 23 | 0.39 | 102 | 1.71 | 25 | 0.42 | 23 | 0.39 | 1,038 | 17.42 | 377 | 6.33 |
| | **2003-2007** | 6,480,899 | 33 | 0.51 | 100 | 1.54 | 15 | 0.23 | 29 | 0.45 | 1,014 | 15.65 | 414 | 6.39 |
| | **2008-2012** | 7,200,600 | 52 | 0.72 | 132 | 1.83 | 20 | 0.28 | 35 | 0.49 | 913 | 12.68 | 373 | 5.18 |
| | **TOTAL** | **42,769,785** | **170** | **0.40** | **668** | **1.56** | **106** | **0.25** | **147** | **0.34** | **8,019** | **18.75** | **3,193** | **7.47** |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **All** | **All** | **85,024,190** | **998** | **1.17** | **2,018** | **2.37** | **183** | **0.22** | **368** | **0.43** | **8,019** | **18.75** | **11,627** | **13.67** |

Abbreviations: SCC = squamous cell carcinoma; HNC = head and neck squamous cell carcinoma; ICC = invasive cervical cancer; n = number of cases; cIR=crude incidence rate

[1] Non-age-standardized incidence per 100,000 person-years; [2] Only women are included in the person-years denominator when calculating incidence of ICC

**Table 6-2.** Age-standardized incidence rates (ASR) per 100,000 person-years for each cancer, overall and by gender, from 1968 to 2012 in Singapore

| | Total | | Men | | Women | | Incidence rate ratio (95%CI) |
|---|---|---|---|---|---|---|---|
| **Cancer type** | **No. of cases** | **ASR[1]** | **No. of cases** | **ASR[1]** | **No. of cases** | **ASR[1]** | **Men : Women** |
| **Oropharyngeal SCC** | 998 | 1.38 | 828 | 2.44 | 170 | 0.44 | 5.54 (4.69-6.54) |
| **Non-oropharyngeal HNC** | 2,018 | 2.77 | 1,350 | 3.91 | 668 | 1.74 | 2.25 (2.05-2.47) |
| **Anal SCC** | 183 | 0.26 | 77 | 0.23 | 106 | 0.29 | 0.80 (0.59-1.07) |
| **Anal non-SCC** | 368 | 0.52 | 221 | 0.67 | 147 | 0.40 | 1.69 (1.37-2.09) |
| **Invasive cervical cancer** | 8,019 | 19.92 | NA | NA | 8,019 | 19.92 | NA |
| **Nasopharyngeal** | 11,627 | 14.06 | 8,434 | 20.63 | 3,193 | 7.64 | 2.70 (2.59-2.81) |
| **Oropharyngeal & Anal SCC** | **1,181** | **1.64** | **905** | **2.67** | **276** | **0.73** | **3.68 (3.21-4.21)** |
| **HPV-associated cancers[2]** | **9,200** | **11.83** | **905** | **2.67** | **8,295** | **20.65** | **0.13 (0.12-0.14)** |

Abbreviations: CI = confidence interval; non-OP HNC = non-oropharyngeal head and neck squamous cell carcinoma; SCC = squamous cell carcinoma

[1] Age-standardized incidence rates per 100,000 person-years**;** Age-standardization was done using the direct method and based on the WHO world standard population

[2] HPV-associated cancers include oropharyngeal SCC, anal SCC and invasive cervical cancer.

**Table 6-3.** Age-standardized incidence rates (ASR) per 100,000 person-years for each cancer, by gender and ethnicity, from 1968 to 2012 in Singapore

| | Men | | | | | | Women | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Chinese | | Malay | | Indian | | Chinese | | Malay | | Indian | |
| **Cancer type** | **No. of cases** | **ASR[1]** | **No. of cases** | **ASR[1]** | **No. of cases** | **ASR[1]** | **No. of cases** | **ASR[1]** | **No. of cases** | **ASR[1]** | **No. of cases** | **ASR[1]** |
| **Oropharyngeal SCC** | 671 | 2.53 | 26 | 0.72 | 115 | 3.64 | 140 | 0.44 | 11 | 0.31 | 17 | 0.96 |
| **Non-oropharyngeal HNC** | 963 | 3.56 | 78 | 2.03 | 283 | 9.12 | 453 | 1.42 | 67 | 1.67 | 142 | 7.51 |
| **Anal SCC** | 48 | 0.19 | 15 | 0.19 | 13 | 0.39 | 92 | 0.29 | 15 | 0.18 | 5 | 0.31 |
| **Anal non-SCC** | 181 | 0.70 | 20 | 0.55 | 17 | 0.57 | 128 | 0.41 | 6 | 0.41 | 4 | 0.20 |
| **Invasive cervical cancer** | NA | NA | NA | NA | NA | NA | 6,969 | 21.29 | 645 | 14.17 | 308 | 13.67 |
| **Nasopharyngeal** | 7,886 | 24.78 | 440 | 9.54 | 54 | 1.53 | 3,026 | 9.01 | 137 | 2.82 | 14 | 0.69 |
| **Oropharyngeal & Anal SCC** | **719** | **2.72** | **41** | **1.11** | **128** | **4.03** | **232** | **0.73** | **26** | **0.49** | **22** | **1.27** |
| **HPV-associated cancers[2]** | **719** | **2.72** | **41** | **1.11** | **128** | **4.03** | **7,201** | **22.03** | **671** | **14.67** | **330** | **14.93** |

Abbreviations: non-OP HNC = non-oropharyngeal head and neck squamous cell carcinoma; SCC = squamous cell carcinoma

[1] Age-standardized incidence rates per 100,000 person-years**;** Age-standardization was done using the direct method and based on the WHO world standard population

[2] HPV-associated cancers include oropharyngeal SCC, anal SCC and invasive cervical cancer.

**Table 6-4.** Trends in crude and age-standardized invasive cervical cancer incidence (ICC) over time, by ethnicity, from 1968 to 2012 in Singapore

| | Chinese | | | | Malay | | | | Indian | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Year of diagnosis** | **Person-years** | **n** | **cIR[1]** | **ASR[2]** | **Person-years** | **n** | **cIR[1]** | **ASR[2]** | **Person-years** | **n** | **cIR[1]** | **ASR[2]** |
| **1968-1972** | 1,992,400 | 527 | 26.45 | 30.80 | 314,800 | 45 | 14.29 | 18.24 | 117,200 | 26 | 22.18 | 44.79 |
| **1973-1977** | 2,421,200 | 588 | 24.29 | 29.46 | 383,500 | 43 | 11.21 | 13.93 | 151,800 | 37 | 24.37 | 41.83 |
| **1978-1982** | 2,826,600 | 639 | 22.61 | 27.98 | 451,100 | 49 | 10.86 | 15.04 | 188,700 | 54 | 28.62 | 47.22 |
| **1983-1987** | 3,262,400 | 801 | 24.55 | 29.37 | 529,600 | 56 | 10.57 | 14.66 | 242,500 | 36 | 14.85 | 20.52 |
| **1988-1992** | 3,733,100 | 878 | 23.52 | 26.90 | 598,300 | 82 | 13.71 | 18.48 | 298,170 | 31 | 10.40 | 15.00 |
| **1993-1997** | 4,266,700 | 994 | 23.30 | 24.94 | 660,200 | 90 | 13.63 | 16.93 | 354,800 | 38 | 10.71 | 14.89 |
| **1998-2002** | 4,736,120 | 912 | 19.26 | 19.17 | 713,753 | 79 | 11.07 | 11.98 | 422,463 | 39 | 9.23 | 11.70 |
| **2003-2007** | 5,080,665 | 866 | 17.05 | 15.76 | 781,445 | 104 | 13.31 | 14.13 | 487,817 | 24 | 4.92 | 5.96 |
| **2008-2012** | 5,529,500 | 764 | 13.82 | 12.23 | 862,300 | 97 | 11.25 | 11.04 | 582,400 | 23 | 3.95 | 4.71 |
| **TOTALS** | **33,848,684** | **6,969** | **20.59** | **21.29** | **5,294,998** | **645** | **12.18** | **14.17** | **2,845,850** | **308** | **10.82** | **13.67** |

[1] cIR = Crude (non-age standardized) incidence per 100,000 person-years

[2] ASR = Age-standardized incidence per 100,000 person-years

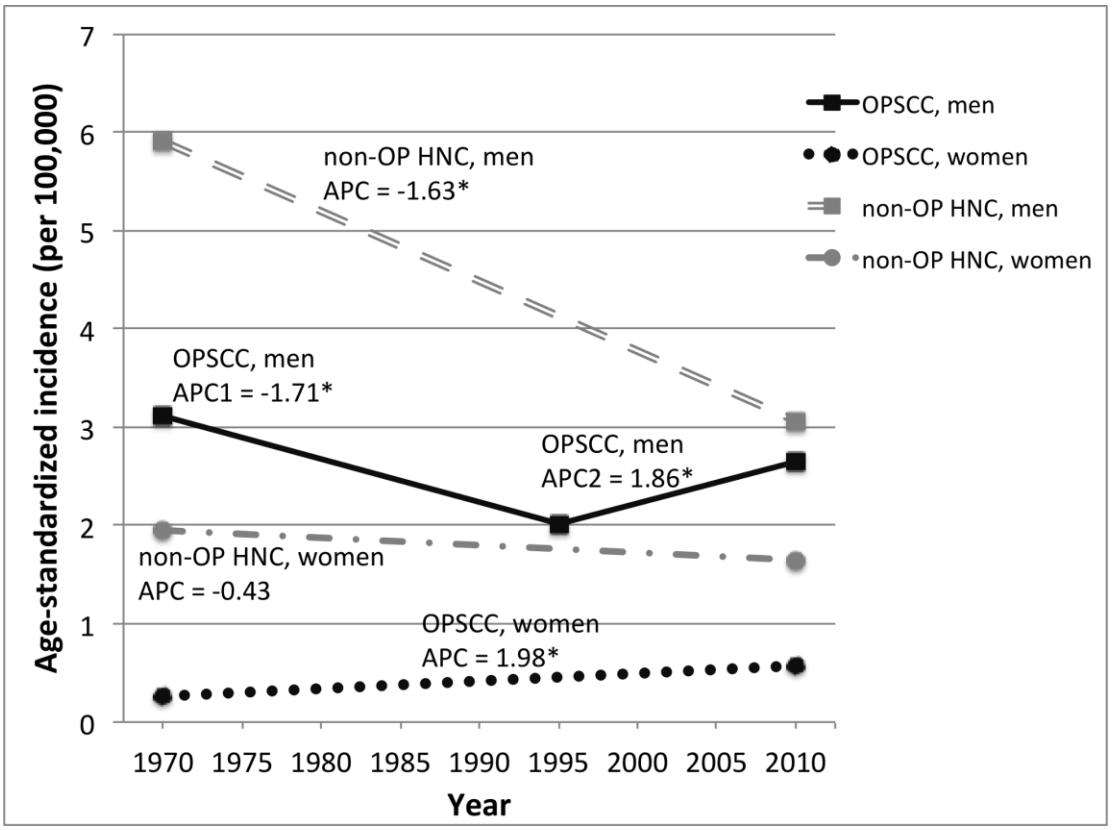
**Table 6-5.** Trends in crude and age-standardized oropharyngeal squamous cell carcinoma (OPSCC) incidence over time, by ethnicity and gender, from 1968 to 2012 in Singapore

| Gender | Year of diagnosis | Chinese | | | | Malay | | | | Indian | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Person-years | n | cIR[1] | ASR[2] | Person-years | n | cIR[1] | ASR[2] | Person-years | n | cIR[1] | ASR[2] |
| **Men** | **1968-1972** | 1,946,200 | 39 | 2.00 | 2.82 | 339,300 | 3 | 0.88 | 1.77 | 265,400 | 16 | 6.03 | 8.54 |
| | **1973-1977** | 2,367,600 | 44 | 1.86 | 2.78 | 406,100 | 3 | 0.74 | 2.16 | 265,800 | 11 | 4.14 | 5.27 |
| | **1978-1982** | 2,775,000 | 40 | 1.44 | 2.27 | 469,900 | 2 | 0.43 | 0.67 | 276,580 | 13 | 4.70 | 6.70 |
| | **1983-1987** | 3,210,000 | 52 | 1.62 | 2.47 | 542,500 | 1 | 0.18 | 0.26 | 315,600 | 8 | 2.53 | 2.15 |
| | **1988-1992** | 3,676,900 | 56 | 1.52 | 2.17 | 611,800 | 6 | 0.98 | 1.86 | 366,300 | 12 | 3.28 | 3.73 |
| | **1993-1997** | 4,152,600 | 63 | 1.52 | 1.97 | 661,600 | 2 | 0.30 | 0.49 | 408,600 | 10 | 2.45 | 2.52 |
| | **1998-2002** | 4,544,901 | 101 | 2.22 | 2.71 | 700,597 | 5 | 0.71 | 1.00 | 467,146 | 11 | 2.35 | 2.73 |
| | **2003-2007** | 4,820,243 | 120 | 2.49 | 2.64 | 756,117 | 2 | 0.26 | 0.19 | 522,110 | 14 | 2.68 | 3.39 |
| | **2008-2012** | 5,195,500 | 156 | 3.00 | 2.75 | 827,400 | 2 | 0.24 | 0.23 | 642,800 | 20 | 3.11 | 3.81 |
| | **TOTAL** | **32,688,944** | **671** | **2.05** | **2.53** | **5,315,314** | **26** | **0.49** | **0.72** | **3,530,336** | **115** | **3.26** | **3.64** |
| **Women** | **1968-1972** | 1,992,400 | 3 | 0.15 | 0.18 | 314,800 | 0 | 0.00 | 0.00 | 117,200 | 1 | 0.85 | 5.38 |
| | **1973-1977** | 2,421,200 | 4 | 0.17 | 0.19 | 383,500 | 0 | 0.00 | 0.00 | 151,800 | 0 | 0.00 | 0.00 |
| | **1978-1982** | 2,826,600 | 11 | 0.39 | 0.48 | 451,100 | 1 | 0.22 | 0.28 | 188,700 | 3 | 1.59 | 3.00 |
| | **1983-1987** | 3,262,400 | 9 | 0.28 | 0.30 | 529,600 | 1 | 0.19 | 0.25 | 242,500 | 0 | 0.00 | 0.00 |
| | **1988-1992** | 3,733,100 | 11 | 0.29 | 0.34 | 598,300 | 1 | 0.17 | 0.40 | 298,170 | 2 | 0.67 | 1.65 |
| | **1993-1997** | 4,266,700 | 8 | 0.19 | 0.21 | 660,200 | 3 | 0.45 | 0.69 | 354,800 | 4 | 1.13 | 1.59 |
| | **1998-2002** | 4,736,120 | 18 | 0.38 | 0.39 | 713,753 | 1 | 0.14 | 0.12 | 422,463 | 2 | 0.47 | 0.62 |
| | **2003-2007** | 5,080,665 | 29 | 0.57 | 0.51 | 781,445 | 2 | 0.26 | 0.34 | 487,817 | 2 | 0.41 | 0.65 |
| | **2008-2012** | 5,529,500 | 47 | 0.85 | 0.71 | 862,300 | 2 | 0.23 | 0.27 | 582,400 | 3 | 0.52 | 0.66 |
| | **TOTAL** | **33,848,685** | **140** | **0.41** | **0.44** | **5,294,998** | **11** | **0.21** | **0.31** | **2,845,850** | **17** | **0.60** | **0.96** |

[1] cIR = Crude (non-age standardized) incidence per 100,000 person-years

[2] ASR = Age-standardized incidence per 100,000 person-years

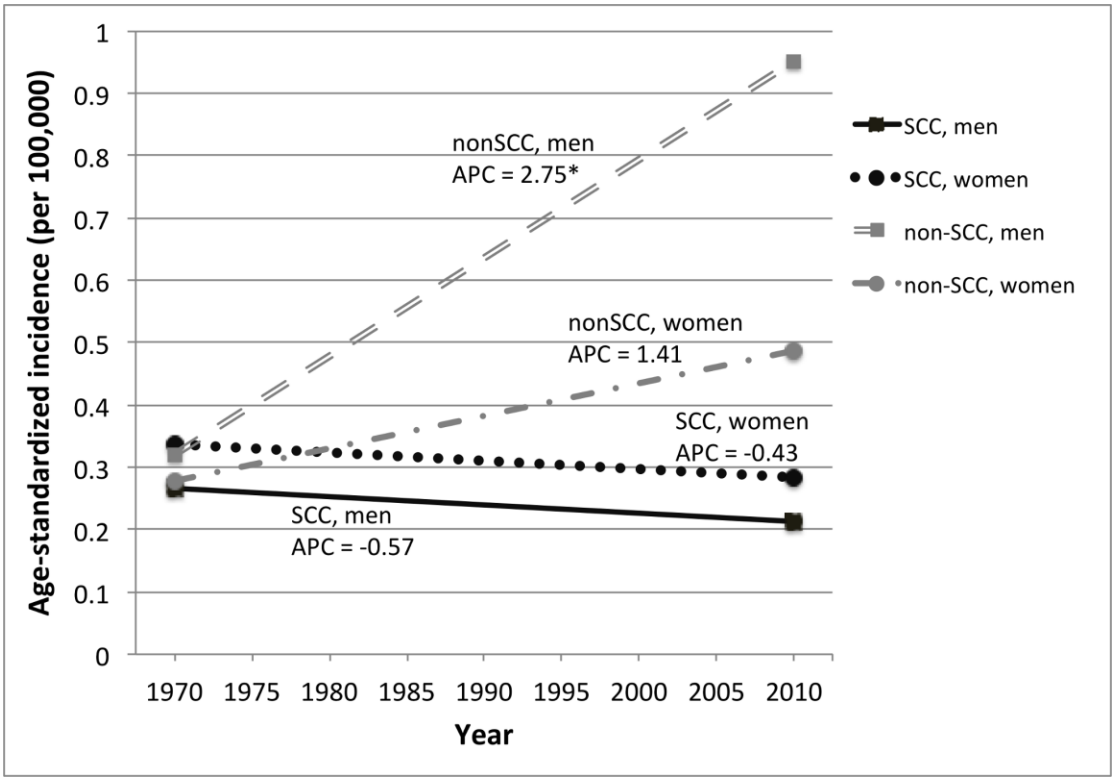
**Figure 6-1.** Incidence of oropharyngeal and non-oropharyngeal head and neck squamous cell carcinomas in Singapore from 1968 to 2012, by gender[1]




Abbreviations: OPSCC = oropharyngeal squamous cell carcinoma; non-OP HNC = non-oropharyngeal head and neck squamous cell carcinoma

[1] Incidence trends are based on incidence rates for 5-year time periods that were age-adjusted to the WHO standard population. Annual percent change (APC) for and are calculated using Joinpoint regression analysis. APC = annual percent change. An asterisk (*) indicates an APC value that is statistically significant at $p<0.05$.

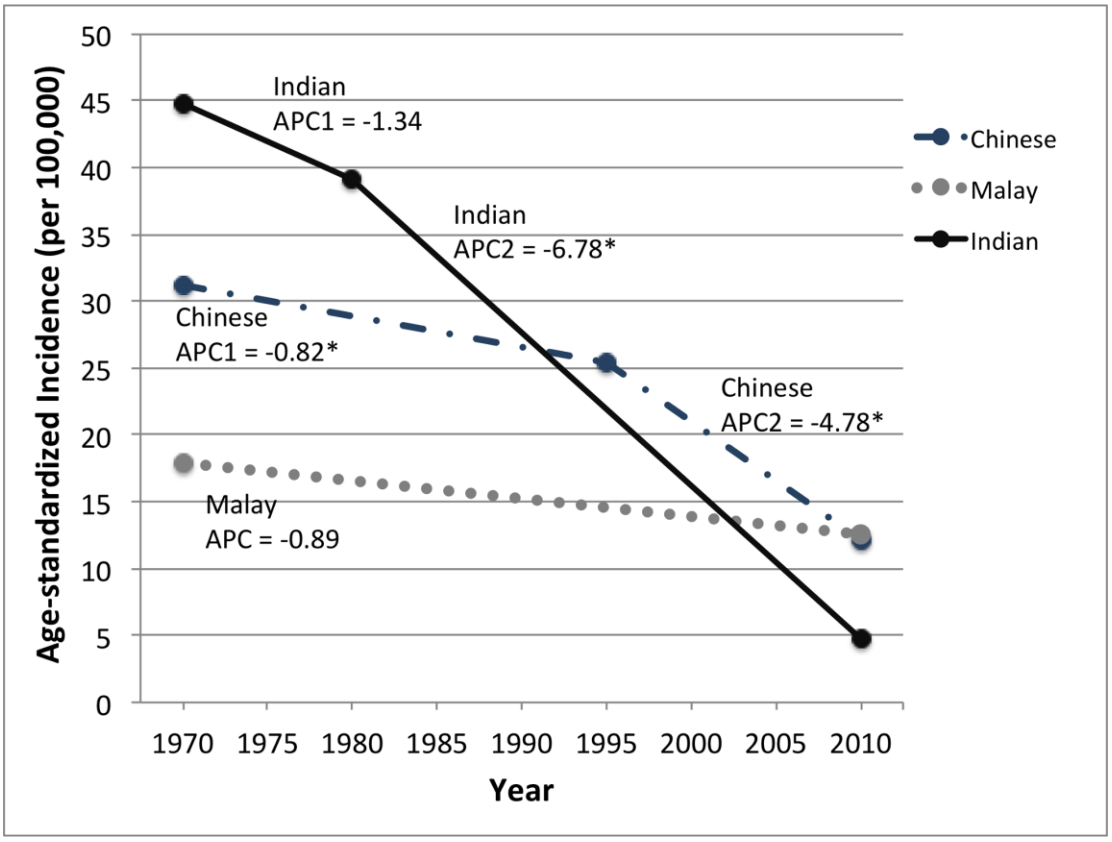
**Figure 6-2.** Incidence of invasive anal cancer in Singapore from 1968 to 2012, by gender and histology[1]




Abbreviations: SCC = squamous cell carcinoma, non-SCC = non-squamous cell carcinoma

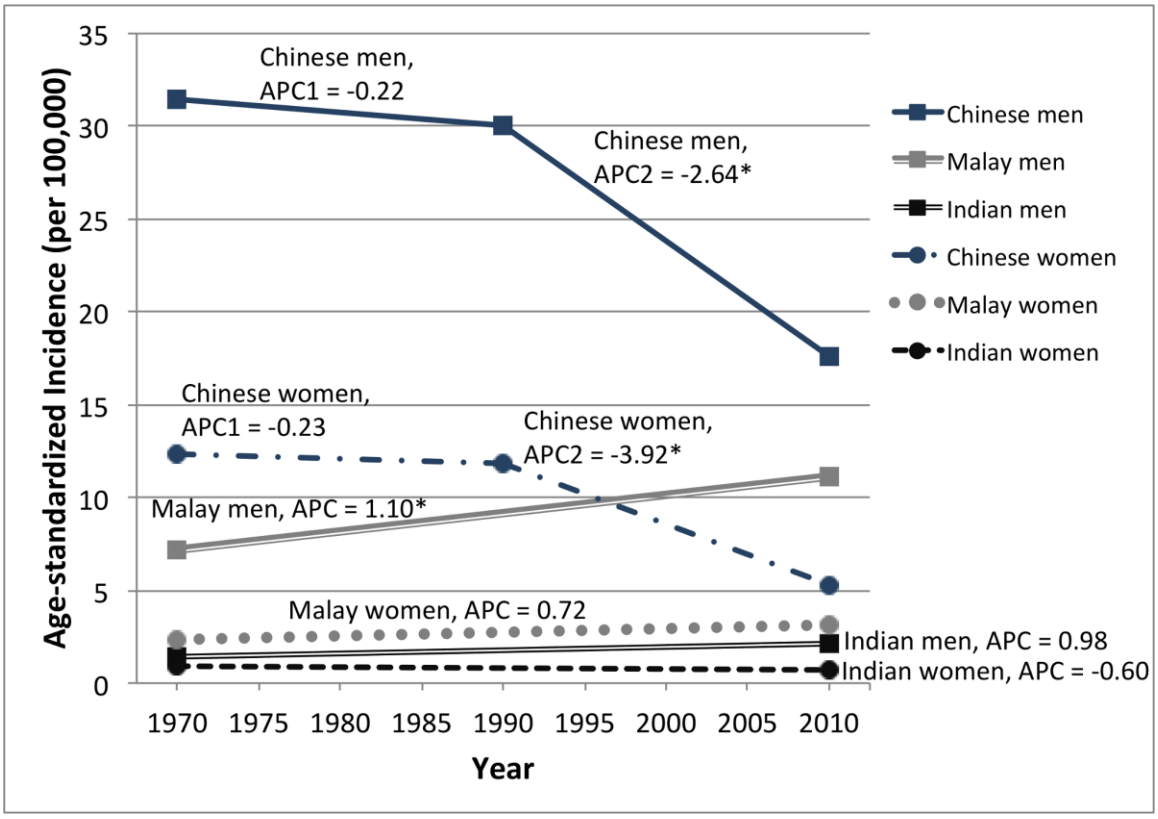
[1] Incidence trends are based on incidence rates for 5-year time periods that were age-adjusted to the WHO standard population and are calculated using Joinpoint regression analysis. APC = annual percent change. An asterisk (*) indicates an APC value that is statistically significant at $p<0.05$.

**Figure 6-3.** Incidence of invasive cervical cancer in Singapore from 1968 to 2012, by ethnicity[1]




[1] Incidence trends are based on incidence rates for 5-year time periods that were age-adjusted to the WHO standard population and are calculated using Joinpoint regression analysis. APC = annual percent change. An asterisk (*) indicates an APC value that is statistically significant at $p<0.05$.

**Figure 6-4.** Incidence of nasopharyngeal cancer in Singapore from 1968 to 2012, by gender and ethnicity[1]




[1] Incidence trends are based on incidence rates for 5-year time periods that were age-adjusted to the WHO standard population and are calculated using Joinpoint regression analysis. APC = annual percent change. An asterisk (*) indicates an APC value that is statistically significant at $p<0.05$.

# Chapter 7. Dissertation overview and summary of key findings

The purpose of this dissertation research was to investigate risk factors for oral HPV persistence and HPV-associated cancers. Epidemiologic and molecular evidence strongly link oral HPV infection with oropharyngeal squamous cell carcinoma (OPSCC). Persistent oral HPV infection is thought to be a precursor to OPSCC, but unlike HPV infection at other anatomic sites, research on oral HPV persistence has been limited. Initial evidence suggests that oral HPV natural history may be similar to that of cervical HPV, but that there may also be important differences. Additionally, epidemiologic data indicate differences in oral HPV and OPSCC incidence and trends by age, biologic sex, geography and race/ethnicity. The reasons for these disparities are unclear and research has primarily been in Western countries, with limited data from Asian populations.

The goals of this research were to examine the association of serum cytokines (Chapter 3), recreational drug use (Chapter 4) and medication use (Chapter 5) with oral HPV clearance. In each of these studies, we focused on HIV-infected individuals and at-risk HIV-negative individuals, populations with high oral HPV prevalence. To contribute Asian data on HPV-associated cancer incidence trends, we described the epidemiology of OPSCC and other potentially HPV-associated cancers, and examined population subgroups at increased risk in Singapore (Chapter 6).

In Chapter 3, we presented results of our study on the association of serum cytokines (TNF-α, IL-6, IL-8, IFN-γ, IL-1β, IL-2, IL-4, IL-10, IL-12 and IL-13) with oral HPV clearance. The conceptual framework for this research was guided by our

knowledge of the cervical HPV model. Select pro-inflammatory cytokines have been associated with reduced cervical HPV clearance, but the association of cytokines with oral HPV has not been previously investigated. Participants were enrolled in the "Persistence of Oral Papillomavirus Study" (POPS), and followed semi-annually. At each visit, oral rinse samples were collected for HPV DNA testing and blood samples were collected for cytokine testing. We found that while most cytokines were not associated with oral HPV clearance, higher concentrations of TNF-α, a pro-inflammatory cytokine, were associated with reduced clearance of oral HPV infection, and this association appeared to be stronger in men than women. This result is consistent with previous research which has suggested that higher pro-inflammatory cytokine concentrations, particularly TNF-α, may be associated with persistent cervical HPV. Furthermore, while previous studies restricted the study population to HIV-uninfected participants,[47-49,52] the association between TNF-α and clearance in our study remained similar after adjusting for HIV infection and CD4 T cell count, suggesting that the relationship between TNF-α and oral HPV may be independent of HIV-related immunosuppression.

In Chapter 4, we present results of a study in the POPS cohort investigating the association of recreational drug use with oral HPV clearance. Recreational drugs have immunomodulatory effects but their role in oral HPV natural history has not been previously explored. We collected semi-annual data on participants' use of alcohol, cigarette, marijuana, crack, cocaine, heroin, amphetamines, ecstasy/club drugs and speedball; and in men only, poppers and sexual performance-enhancing drugs. We found that while most drugs were not associated with oral HPV clearance, cocaine use in the 6-

month time period before first detection of oral HPV infection may be an important risk factor for reduced oral HPV clearance.

In Chapter 5, we present results of a study in the POPS cohort investigating the association of medication use with oral HPV clearance. Given the increasing prevalence of polypharmacy in the population aging with HIV, and the known immunomodulatory effects of several commonly used medications, we undertook this research to evaluate whether medication use may affect oral HPV clearance. Data were collected on use of: antiasthmatics, antidepressants, antihypertensives, antipsychotics, anxiolytics and sedatives, cholesterol-lowering medications, diabetes medications, hormones, non-steroidal anti-inflammatory drugs (NSAIDs) and erectile dysfunction medications. We found that participants using antipsychotic medications had significantly reduced oral HPV clearance, and that the effects were stronger in participants with HIV infection. Antidepressants and anxiolytics/sedatives were also associated with reduced clearance, although the association was not as strong as with antipsychotics. Importantly, combined use of 2 or more of these 3 medications (antipsychotics, antidepressants, anxiolytics/sedatives) was associated with elevated risk for persisting oral HPV. Each of these medications are prescribed for conditions that have immunomodulatory effects, so characteristics of the illness may have partially contributed to the reduced oral HPV clearance observed in this study.

Chapter 6 reviews the incidence and temporal trends of potentially HPV-associated cancers in Singapore. Data from 1968-2012 were compiled from the Singapore Cancer Registry, which is representative of the Singapore resident population. We found that the incidence of HPV-associated cancer overall was higher in women than men, due

to the burden of cervical cancer among women, but OPSCC incidence was 5 times higher in men than women. Ethnic differences in incidence of HPV-associated cancer were observed, with higher rates overall among Indian men and among Chinese women than other ethnicities. OPSCC rates increased in both men and women in recent years, similar to trends reported in other countries of comparable socioeconomic status.[2,10,17,18,20,225,226,230-236] In contrast, the incidence of other head and neck cancers (non-oropharyngeal and nasopharyngeal cancer) decreased. Anal cancer was rare. ICC was the most common HPV-associated cancer but declined significantly over the 45 years studied, largely due to the success of Pap screening programs. This research suggests the distribution of HPV-associated cancers across population subgroups has changed over the past few decades, possibly mirroring changes in tobacco and sexual risk factors.

# Chapter 8. Public health implications and directions for future research

This dissertation contributes to the scientific literature on HPV-related OPSCC, an emerging public health issue in several regions of the globe. The studies presented increase our knowledge about immunologic and drug/medication use risk factors that may impact oral HPV natural history, and provide insight on risk factors for HPV-associated cancers.

To our knowledge, our study of serum cytokines and oral HPV clearance is the first to explore the association of circulating immune markers with oral HPV clearance. Since there is neither standardized oral screening, nor easily identifiable precursor lesion for OPSCC,[63,64] biomarkers related to infection duration may help identify oral HPV-infected individuals at increased risk for persistent infection and/or HPV-related OPSCC. Future studies, which include longitudinal cytokine evaluation, are needed to clarify the role of TNF-α in oral HPV natural history. The results of this study contribute evidence that immune differences may explain why some people clear oral HPV while others to do not, but questions remain about the role of oral immunity in oral HPV infection. There is growing interest in the use of salivary sampling for diagnostic, prognostic and health surveillance purposes.[64] While initial studies suggest that serum cytokine measurements correlate with markers detectable in the oral cavity,[82] the use of these markers as indices of oral HPV natural history has not been evaluated. Additional study of immune components in saliva may help clarify the value of salivary measurements in research on

oral HPV and shed useful insight on the role of oral immunity in oral HPV natural history.

This is the first study to report an association of cocaine use with reduced oral HPV clearance. Future studies may consider examining whether the frequency and intensity of cocaine use affects oral HPV clearance in a dose-response fashion, as we were limited in sample size to examine this relationship in our research. The mechanisms by which cocaine use may reduce oral HPV clearance is another potential area for research. Future studies may consider use of direct measures of cocaine exposure in order to reduce biases that could arise from self-reported drug use. Measurement of bezoylecgonine, a cocaine metabolite, in oral fluid has been studied for potential use in non-clinical contexts, including monitoring of drug use in the workplace, criminal justice and point-of-collection testing devices for assessment of driving under the influence.[133] However, while bezoylecgonine is the most promising direct measure of cocaine exposure, it is metabolized within 1 to 2 days and has relatively low concentrations in body fluids.[129,275] Its use in clinical research of oral HPV may be possible as technologies for metabolite detection evolve.[129]

Immunomodulatory medications may also be a risk factor for oral HPV persistence. Our initial results suggest that some medications, particularly those prescribed for neuropsychiatric disorders, may reduce oral HPV clearance. However, illness characteristics are multi-faceted and complex, as are the treatment regimens prescribed to treat them. Additional research on medications subtypes, dosages and frequency of usage will be needed to better characterize the relationship between medication use and oral HPV natural history.

Previous studies have suggested that HIV may be associated with reduced oral HPV clearance, and so in studying risk factors for oral HPV persistence, we considered each potential risk factor separately by HIV status. While there was some suggestion that the effects of cocaine and antipsychotic medication on reduced oral HPV clearance were stronger in HIV-infected individuals as compared to HIV-uninfected individuals, we found no compelling evidence that HIV substantially modified the effects of these potential risk factors. As it is difficult to disentangle the influences of HIV-related immunodeficiency and ongoing immune dysfunction, from the immunomodulatory effects of other exposures, it remains important in future research to examine potential effect modification by HIV status.

The analysis of cancer epidemiology in Singapore increases our understanding of the current burden and trends of HPV-associated cancers, contributing some of the first Asian data on this topic. Although HPV-associated cancer prevention in Singapore has primarily focused on cervical cancer, our study shows for the first time that there is also a burden of potentially HPV-related OPSCC in men, and that the incidence is rising, in contrast to other head and neck cancers. Initial research suggests HPV vaccination will likely also be effective in preventing oral HPV infection and thus could impact OPSCC rates.[267,268] Current HPV prevention efforts in Singapore focus primarily on cervical cancer, but should prevention efforts expand to include HPV-related OPSCC, it will be important to monitor changes from the baseline epidemiologic profile we report in our study. The 45-year period that we studied encompassed Singapore's rapid transition from a low-income to a high-income country. This economic transition was accompanied by changing disease epidemiology, with cancers replacing infectious diseases as the primary

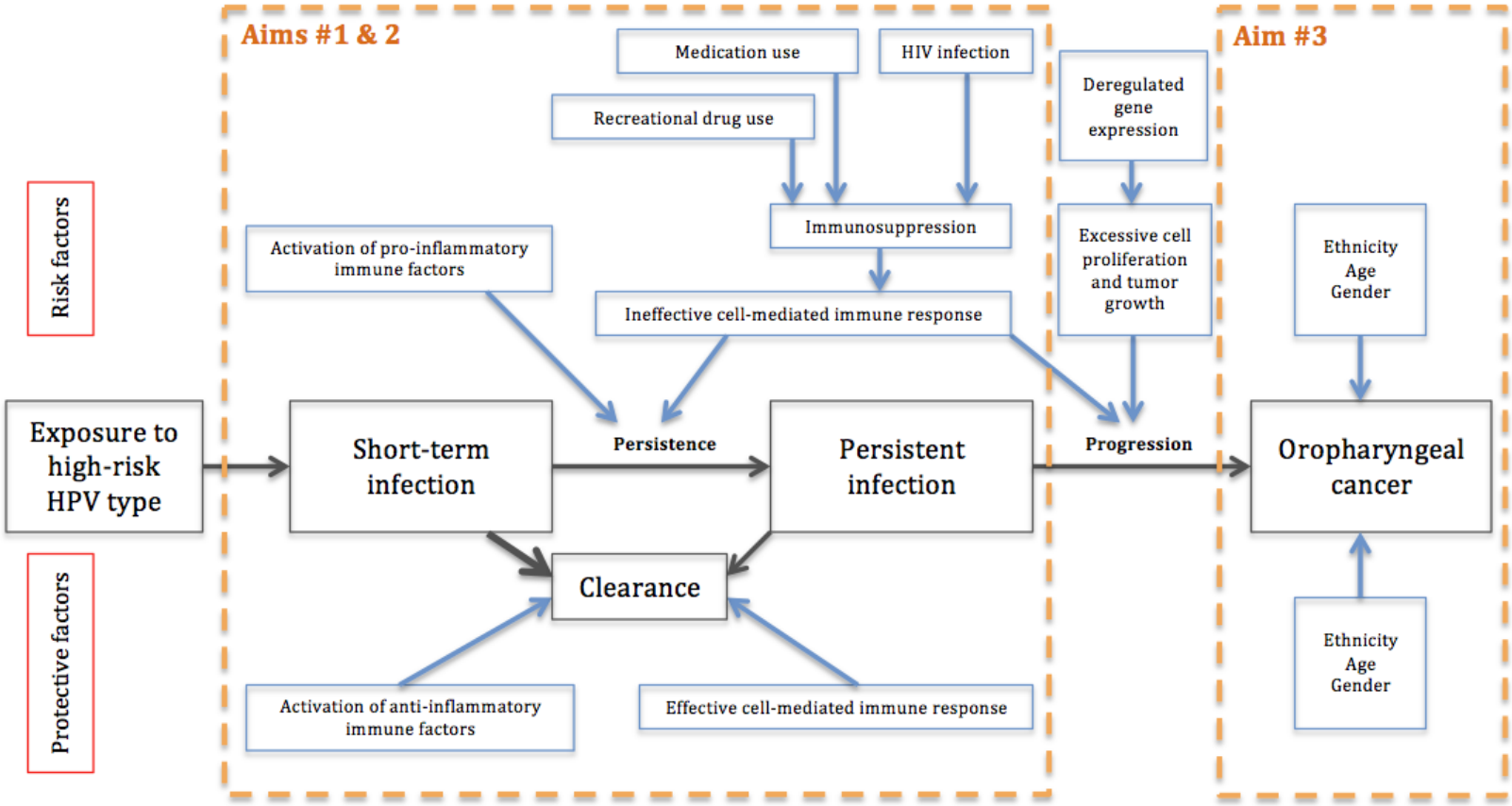
cause of mortality.[276] The incidence and trends of the cancers we describe during Singapore's economic transition may be relevant for the future cancer patterns in other economically rising countries in Asia. Patients with HPV-associated HNSCC have better survival and treatment outcomes than patients with HPV-negative HNSCC,[1] and thus, shifts in HNSCC mortality may also be observed along with the changing etiologic profile of these cancers.

Our study is one of the first investigations of disparities in HPV-associated cancer incidence in the major ethnic groups in Singapore (ie. Chinese, Malay, Indian). The results of the research may be useful to inform cancer prevention strategies for Singaporean subgroups at highest risk for HPV-associated cancers. In order to develop appropriate public health prevention programs and interventions, future research exploring the underlying reasons for disparities in cancer incidence and trends will be important. Currently, HPV tumor typing is not routinely done in Singapore and so we could not confirm the contribution of HPV infection to the incidence and trends observed. Future studies, which include HPV tumor status, may be able to more clearly distinguish tobacco/alcohol- vs. HPV-related OPSCC, especially if trends in these etiologically distinct cancers continue to diverge.

The findings presented in this dissertation contribute to explaining why the burden of OPSCC and other HPV-associated cancers are disproportionately high in some population subgroups. We have presented new insights on immunologic and drug/medication use factors that may influence oral HPV persistence and have uncovered new questions for future study. As our understanding of oral HPV persistence improves,

these initial findings may help shape cancer prevention strategies in HIV-infected individuals and other groups at high risk for HPV infection.

# Appendix A. Conceptual framework for factors associated with oral HPV persistence and oropharyngeal cancer



# Appendix B. MACS drug use questionnaire

[Modified from MACS Follow-up Visit form, Section 4]

I am going to ask you a series of questions about specific behaviors, including cigarette smoking, alcohol use, sexual behavior, and recreational drug use.

Now I have some questions about cigarette smoking.

Have you ever smoked cigarettes?
- o No
- o Yes

Do you smoke cigarettes now? (As of one month ago)?
- o No
- o Yes
- o Occasionally (less than one cigarette per day)

How many packs do you usually smoke per day?
- o Less than ½ pack
- o At least ½ pack, but less than one pack per day
- o At least 1 but less than 2 packs
- o 2 or more packs per day

The next set of questions are about alcoholic beverages. They may seem similar, but they are asked in a slightly different way.
Please answer each of the following questions for the past 6 months.

How often have you had drinks containing alcohol?
- o Never
- o Less than monthly
- o Monthly
- o Weekly
- o Daily or almost daily

During the past 6 months, how many drinks containing alcohol have you had on a typical day when you are drinking? (A "drink" is defined as one 12-ounce beer, one 5-ounce glass of wine, or one mixed drink with 1 and 1/2 ounces of 80-proof hard liquor.)
- o None
- o 1 or 2
- o 3 or 4
- o 5 or 6
- o 7 to 9
- o 10 or more

During the past 6 months, how often have you had six or more drinks on one occasion? (A "drink" is defined as one 12-ounce beer, one 5-ounce glass of wine, or one mixed drink with 1 and 1/2 ounces of 80-proof hard liquor.)

- Never
- Less than monthly
- Monthly
- Weekly
- Daily or almost daily

Now let's talk about other drugs you may have used. As I read each one, please tell me whether you used it even once since your last visit [in (MONTH, YEAR)]?

How about (EACH) Have you (taken/used) any since your last visit [in (MONTH, YEAR)]?

1. Pot, marijuana or hash
2. "Poppers" like nitrite inhalants (amyl, butyl or isopropyl nitrites)
3. Crack or cocaine that you smoke
4. Other forms of cocaine
5. Speed, meth or ice
6. Heroin
7. Speedball (heroin and cocaine together)
8. Ecstasy, XTC, X or MDMA
9. Sexual performance enhancing drugs other than those prescribed by a medical provider for diagnosed erectile dysfunction (Show list of performance enhancing drugs to prompt and assist with recall)
10. Other kinds of street/club drugs

How often did you (use/take) (DRUG) since your last visit [in (MONTH, YEAR)]?

- Daily
- Weekly
- Monthly
- Less often

How did you (use/take) (DRUG) since your last visit [in (MONTH, YEAR)]? [Mark all that apply]

- Snorted
- Swallowed
- Put in anus ("booty bumped")
- Smoked
- Injected (intravenous use)

# Appendix C. WIHS drug use questionnaire

[Modified from WIHS "F24Beh: Alcohol, drug use and sexual behavior" interviewer script and questionnaire]

Now I am going to ask you some personal questions about your cigarette and alcohol use, if any.

Since your study visit on MM/DD/YY have you smoked cigarettes?

- No
- Yes

How many cigarettes, on the average, do you smoke each day?

Since your (MONTH) study visit, did you drink beer, wine, hard liquor or any other alcoholic beverages?

- No
- Yes

Now I'm going to ask you about the alcoholic beverages you drank since your (MONTH) study visit. By "a drink" I mean one can, bottle or glass of beer, a glass of wine, a shot of liquor, a mixed drink with that amount of liquor, or any other alcoholic beverage.

a. Since your (MONTH) study visit, how often did you have a drink containing alcohol?
    - 1-2 days a week
    - 1-2 times a month
    - About once a month
    - 6-11 times a year
    - 1-5 times a year

b. Since your (MONTH) study visit, on a day when you drank any alcoholic beverages, about how many did you USUALLY have altogether?
    - 1 drink
    - 2 drinks
    - 3 drinks
    - 4 drinks
    - 5 drinks
    - 6 drinks
    - 7 to 9 drinks
    - 10 or more drinks

Now I will ask you a few questions about drug use. Your answers are strictly confidential.

Since your (MONTH) study visit, have you used marijuana, either medical or recreational, cocaine, crack, heroin, methamphetamine, hallucinogens, club drugs, or any other illicit or recreational drugs? If yes, continue with questionnaire.

Since your (MONTH) study visit, **have you used marijuana or hash** to get high, for medical reasons, or both?

On average, how often did you use marijuana or hash recreationally since your (MONTH) study visit?

- Less than once a month
- At least once a month, but less than once a week
- Once a week
- 2 – 3 times a
- 4 – 6 times a week
- Once a day
- More than once a day
- Never

(Since your (MONTH) study visit), **have you smoked crack**?
On average, how often have you smoked crack since your (MONTH) study visit?

- Less than once a month
- At least once a month, but less than once a week
- Once a week
- 2 – 3 times a week
- 4 – 6 times a week
- Once a day
- More than once a day

(Since your (MONTH) study visit), **have you injected crack by itself**?

On average, how often have you injected crack since your (MONTH) study visit?

- Less than once a month
- At least once a month, but less than once a week
- Once a week
- 2 – 3 times a week
- 4 – 6 times a week
- Once a day
- More than once a day

(Since your (MONTH) study visit), **have you sniffed, snorted, or smoked cocaine**?
On average, how often have you sniffed, snorted, or smoked cocaine since your (MONTH) study visit?

- Less than once a month

- At least once a month, but less than once a week
- Once a week
- 2 – 3 times a week
- 4 – 6 times a week
- Once a day
- More than once a day

(Since your (MONTH) study visit), **have you injected cocaine by itself**?

On average, how often have you injected cocaine since your (MONTH) study visit?
- Less than once a month
- At least once a month, but less than once a week
- Once a week
- 2 – 3 times a week
- 4 – 6 times a week
- Once a day
- More than once a day

(Since your (MONTH) study visit), **have you sniffed or snorted heroin**?

On average, how often have you sniffed or snorted heroin since your (MONTH) study visit?
- Less than once a month
- At least once a month, but less than once a week
- Once a week
- 2 – 3 times a week
- 4 – 6 times a week
- Once a day
- More than once a day

(Since your (MONTH) study visit), **have you smoked heroin**?

On average, how often have you smoked heroin since your (MONTH) study visit?
- Less than once a month
- At least once a month, but less than once a week
- Once a week
- 2 – 3 times a week
- 4 – 6 times a week
- Once a day
- More than once a day

(Since your (MONTH) study visit), **have you injected heroin by itself**?

On average, how often have you injected heroin since your (MONTH) study visit?
- Less than once a month
- At least once a month, but less than once a week

- Once a week
- 2 – 3 times a week
- 4 – 6 times a week
- Once a day
- More than once a day

(Since your (MONTH) study visit), **have you injected heroin and cocaine together** (speedball)?

On average, how often have you injected heroin and cocaine together (speedball) since your (MONTH) study visit?

- Less than once a month
- At least once a month, but less than once a week
- Once a week
- 2 – 3 times a week
- 4 – 6 times a week
- Once a day
- More than once a day

(Since your (MONTH) study visit), **have you sniffed or smoked methamphetamine** (crank, crystal, tina)?

On average, how often have you sniffed or smoked methamphetamine since your (MONTH) study visit?

- Less than once a month
- At least once a month, but less than once a week
- Once a week
- 2 – 3 times a week
- 4 – 6 times a week
- Once a day
- More than once a day

(Since your (MONTH) study visit), **have you injected methamphetamine** (crank, crystal, tina) **by itself**?

On average, how often have you injected methamphetamine since your (MONTH) study visit?

- Less than once a month
- At least once a month, but less than once a week
- Once a week
- 2 – 3 times a week
- 4 – 6 times a week
- Once a day
- More than once a day

(Since your (MONTH) study visit), **have you used hallucinogens** (such as LSD, PCP, mushrooms, peyote)?

(Since your (MONTH) study visit), **have you used any club drugs, such as ecstasy, ketamine or GHB**?

On average, how often have you used club drugs since your (MONTH) study visit?

- At least once a month, but less than once a week
- Once a week
- 2 – 3 times a week
- 4 – 6 times a week
- Once a day
- More than once a day

(Since your (MONTH) study visit), **have you used amphetamines** (speed, uppers) **in a way that was not prescribed?** Not prescribed means you didn't have a doctor's prescription for the amphetamine, you used more than was prescribed, or you used it to get high.

On average, how often have you used amphetamines in a way that was not prescribed since your (MONTH) study visit?

- Less than once a month
- At least once a month, but less than once a week
- Once a week
- 2 – 3 times a week
- 4 – 6 times a week
- Once a day
- More than once a day

# Appendix D. MACS medication use questionnaire

[Modified from MACS Follow-up Visit form, Section 4]

Now, I have some questions about drugs and medications that you may have taken for other health reasons. These include prescribed medications, over the counter medications, and other medications you took on your own since your last visit [in (MONTH, YEAR)].

How about [EACH]? Have you (taken/used) any since your last visit [in (MONTH, YEAR)]?

1. Steroids that you took by mouth or were injected
2. Thyroid hormone or thyroid medication
3. Other hormones such as anabolic steoroids
4. Tranquilizers or sleeping pills
5. Antidepressants or mood elevators
6. Lithium
7. Viagra, Cialis, Levitra or other drugs that were prescribed by a medical provider to treat erectile dysfunction
8. Aspirin taken three days or more on a weekly basis
9. Medications to lower cholesterol, triglycerides, lipids or blood fat
10. Medications to treat hypertension
11. Medications to treat diabetes

Have you (taken/used) any other medications since your last visit [in (MONTH, YEAR)]?
When specified, what was the name of the (KIND OF DRUG) you took?
What did you take this drug for?

# Appendix E. WIHS medication use questionnaire

[Modified from Form 22 Med: Medication History]

How I am going to ask you a series of questions about medicines that you may have had or taken since your study visit on MM/DD/YY.

Also, if at any point in the interview you wish to stop, let me know.

Finally, I need to re-emphasize that all your answers are confidential, and the responses you provide will in no way affect your clinical care.

Since your (MONTH) study visit, have you taken any of the following hormone replacement therapies (hormones, estrogen, progesterone) for more than one month? These therapies could have been taken in the form of a pill, cream, or patch worn on the skin. Please do not include any hormones taken only to prevent pregnancy; we will discuss those later in the interview.

ESTROGEN: Premarin, Estrace, Estratab, Menest, Ogen, Cenestin, Estraderm, Climara, Menostar, Estrasorb, Alora, Enjuvia, Evamist, Femring, Vivelle-Dot

PROGESTERONE: Provera, Cycrin, Amen, Prometrium, Micronor, Nor-QD

COMBINATION ESTROGEN/PROGESTERONE: Premphase, Prempro, Combipatch, Angeliq, Activella, Prefest

OTHER HRT: Tamoxifen, Raloxifene, testosterone patch or cream, Estratest (combination estrogen/testosterone), birth control pills, Norplant, Ortho Evra (birth control patch), NuvaRing (a vaginal ring containing hormone)

YES .....1
NO .....2

Since your (MONTH) study visit, have you taken any medication for blood pressure or your heart,(such as Amiodarone, Quinidine, Verapamil, hydrochlorothiazide, etc.)?

YES .....1
NO .....2

(Since your (MONTH) study visit), have you taken any medication to lower your cholesterol, triglyceride, or blood lipid level, (such as Lipitor, Pravachol, Zocor, etc.)?
YES .....1
NO .....2

(Since your (MONTH) study visit), have you taken any medication to lower your blood sugar, such as insulin injections or any oral medications?
YES .....1
NO .....2

(Since your (MONTH) study visit), have you taken any medication for psychological conditions or depression, (such as Zyprexa, Zoloft, Celexa, etc.)?
YES .....1
NO .....2

(Since your (MONTH) study visit), have you taken any medication for HIV lipodystrophy or body fat changes related to HIV, such as growth hormones or steroids?
YES .....1
NO .....2

(Since your (MONTH) study visit), have you taken any medication for breathing or lung problems,(such as Singulair, monteleukast, Accolate, zafirlukast, Zyflo, zileutin, Theodur, theophylline, Slophyllin,Slo-bid, Aerolate, or albuterol)?
YES .....1
NO .....2

Since your (MONTH) study visit, have you taken any other prescribed medications not previously mentioned?
YES .....1
NO .....2

# Appendix F. Medication types and classification

| Medication type | Medication class(es) | Examples of medication brand names (generic/drug names in parentheses) |
|---|---|---|
| **Antiasthmatic (non-steroidal)** | Bronchodilators, including<br>1. β-agonists<br>2. Leukotriene modifiers | 1. Ventolin (albuterol), Xopenex (levalbuterol), Servent (salmeterol)<br>2. Singulair (montelukast), Accolate (zafirlukast) |
| **Antiasthmatic (steroidal)** | Inhaled steroids | Advair (fluticasone+salmeterol), Flovent (fluticasone), Symbicort (budesonide with formoterol), Aerobid (flunisolide), Azmacort (triamcinolone) |
| **Antidepressants** | 1. Selective serotonin reuptake inhibitors (SSRIs)<br>2. Serotonin-norepinephrine reuptake inhibitors (SNRIs)<br>3. Norepinephrine reuptake inhibitors (NRIs) | 1. Celexa (citalopram), Paxil (paroxetine), Zoloft (sertraline), Prozac (fluoxetine)<br>2. Cymbalta (duloxetine)<br>3. Wellbutrin (buproprion) |
| **Antihypertensives** | 1. Diuretics<br>2. ACE-inhibitors<br>3. Beta-blocking agents<br>4. Calcium channel blockers<br>5. Other, including anti-adrenergic agents | 1. Microzide (hydrochlorothiazide), Lasix (furosemide), acetazolamide<br>2. Vasotec (enalapril), Prinivil (eisinopril), verapamil<br>3. Lopressor (metoprolol), Coreg (carvedilol), Tenormin (atenolol)<br>4. Procardia (nifedipine), Norvasc (amlodipine)<br>5. Nexiclon (clonidine), hydralazine |
| **Antipsychotics** | 1. Typical<br>2. Atypical | 1. Abilify (aripiprazole), Haldol (haloperidol), lithium<br>2. Risperdal (risperidone), Seroquel (quetiapine) |
| **Anxiolytics/sedatives** | 1. Benzodiazepines<br>2. Other sedatives | 1. Xanax (alprazolam), Valium (diazepam), Ativan (lorazepam), Klonopin (clonazepam) |

| | | |
|---|---|---|
| | | 2. Ambien (zolpidem) |
| **Cholesterol-lowering** | 1) Statins<br>2) Fibrates | Lipitor (atorvastatin), Altocor, Altoprev, Mevacor (all lovastatin), Tricor (fenofibrate), Crestor (rosuvastatin), Pravigard (pravastatin), Vytorin (ezetimibe+simvastatin) |
| **Diabetes** | 1. Insulin and analogues<br>2. Blood glucose lowering drugs, excluding insulins<br>3. Other drugs used in diabetes, including combination drugs | 1. Lantus (glargine), Apidra (insulin glulisine)<br>2. Januvia (sitagliptin), Glucophage (metformin), Glucotrol (glipizide), Diabeta (mlyburide), Glyset (Migitol), Actos (pioglitazone)<br>3. Janumet (sitagliptin+metformin), Glucovance (Glyburide+ Metformin) |
| **Erectile dysfunction (men only)** | PDE 5 inhibitors | Viagra (sildenafil), Cialis (tadalafil), Levitra (vardenafil) |
| **Hormones** | | Levothyroid (levothyroxine), Cytomel (liothyronine), growth hormone, estrogen, testosterone |
| **Non-steroidal anti-inflammatory drugs (NSAIDs)** | Anti-inflammatory | Advil (ibuprofen), Aleve (naproxen), aspirin, naproxen, Motrin (ibuprofen) |

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# Curriculum Vitae


## Jennifer Lam

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### *Education*

2016 **Doctor of Philosophy (Ph.D.) in Global Disease Epidemiology & Control**
Johns Hopkins Bloomberg School of Public Health
Department of International Health
Advisor: Dr. Gypsyamber D'Souza

2009 **Master of Public Health (M.P.H.), Infectious Disease Epidemiology**
Johns Hopkins Bloomberg School of Public Health
Advisor: Dr. William Moss

2003 **Bachelor of Arts (B.A.) in English**
University of California, Berkeley

### *Experiences*

**Collaborator** (7/2015 – present)
Global Burden of Disease Study
Institute for Health Metrics and Evaluation; Seattle, WA

Participate in research network aimed at estimating cause-specific mortality rates and trends by providing consultation as needed

- Critiqued cause of death estimates, with focus on evaluation and interpretation of age- and time-trends of cancer-related mortality in Singapore, China and India
- Reviewed manuscript on global, regional and national life expectancy assessments from 1980-2015

**Research Assistant** (3/2013 – present)
Persistence of Oral Papillomavirus Study
Johns Hopkins School of Public Health; Baltimore, MD

- Conduct statistical analyses on longitudinal data and repeated laboratory measurements to evaluate risk factors for persistent oral HPV infection in individuals with or at risk for HIV

- Evaluate incidence, trends and ethnic differences of HPV- and infection-related cancers in Singapore using cancer registry data

**Primary Instructor** (1/2012, 1/2013)
Intersession Program; *Vaccines and Child Health* course
Johns Hopkins University; Baltimore, MD

Designed and developed a course for college freshmen, with the goal of teaching the science of immunization while strengthening students' connection to public health work in Baltimore City.

- Conduct lectures on vaccine-preventable diseases, surveillance and outbreak investigation, immunology, local child health initiatives, and strategies for maintaining high vaccination coverage
- 30 undergraduates per session

**Vaccine Research Intern** (9/2012 – 12/2012)
Department of Immunization, Vaccines and Biologicals
World Health Organization; Geneva, Switzerland

Investigated the use of global surveillance systems to support the introduction of new and underutilized vaccines.

- Assessed global surveillance results and trends for invasive bacterial diseases (IBD) from 2008-2011 in countries at various stages of polysaccharide conjugate vaccine introduction
- Compiled WHO/UNICEF Joint Reporting Form data and evaluated use of meningitis surveillance indicators for summarizing country- and WHO region-specific IBD surveillance performance
- Conducted literature review of methods for assessing impact of HPV vaccines

**Epidemiologist** (8/2009 – 7/2011)
Center for Immunization; Infectious Disease and Environmental Health Administration
Maryland Department of Health & Mental Hygiene; Baltimore, MD

Collaborated with team, and worked with liaisons at local health centers, to track and reduce vaccine-preventable diseases (VPDs) in Maryland and to improve vaccination rates in school-aged children and the general population.

- Served as VPD contact person for the State's 24 jurisdictions; provided guidance on infectious disease case investigations, outbreak control and recommended immunizations
- Developed State-wide protocols for investigation of measles, mumps and pertussis
- Created biweekly reports on the distribution of H1N1 influenza vaccine to high-risk populations
- Analyzed vaccination coverage data on children in Kindergarten through Grade 12

**Consultant** (1/2009 – 11/2009)
Neonatal Health in Complex Humanitarian Emergencies Project
Save the Children & Johns Hopkins Bloomberg School of Public Health

Joined study team working to document the current state of neonatal healthcare in emergency situations and to explore interventions feasible for implementation at community and primary health facility levels.

- Identified evidence-based neonatal care methods that can be adapted for use in crisis situations
- Developed & implemented survey for administrators and policy-makers at key humanitarian organizations in 34 countries to evaluate strengths and barriers to program development

**TB Control Intern** (10/2008 – 5/2009)
Division of TB Control, Refugee and Migrant Health
Maryland Department of Health & Mental Hygiene; Baltimore, MD

Selected for internship investigating the relationship between TNF-alpha blockers and TB to provide recommendations to physicians in Maryland with patients who have latent TB and require TNF-alpha blocker medications.

- Wrote research proposal based on Maryland TB surveillance data and recent literature
- Created and implemented survey to evaluate TB screening practices of physicians
- Compiled and analyzed data; presented report of findings and recommendations

**Contributing Author** (1/2006 – 5/2008)
Online Collaborative Training for AIDS Vaccine Evaluation (OCTAVE) Project
Joined the OCTAVE team, an NIH-funded initiative aimed at using e-Learning methods to train new investigators in resource-poor settings. Worked with team members to create high-quality online curriculum for investigative teams in over 30 countries preparing for or actively conducting clinical trials of HIV vaccine candidates.

- Designed and conducted surveys to ensure training curricula focused on topics relevant to learners in diverse and geographically dispersed areas
- Wrote curricula on DNA vaccinology and use of adjuvants in HIV vaccines
- Created web-based tutorials to assist users in navigating training materials

**Research Associate** (1/2004 – 5/2008)
AIDS Office/HIV Research Department
San Francisco Department of Public Health

- Managed over 200 volunteers in 18 HIV vaccine and prevention clinical trials
- Conducted informed consent process; enrolled eligible candidates into trials according to study inclusion/exclusion criteria
- Provided pre- and post-test HIV counseling; collected and processed blood specimens
- Administered study questionnaires; assisted with data management and QA procedures

**Coordinator** (2/2002 – 5/2003)
Hepatitis Testing, Education and Vaccination Project
Berkeley Free Clinic; Berkeley, CA

- Initiated hepatitis B outreach project for Asian American and immigrant populations

- Organized group of 25 hepatitis counselors

### *Honors and awards:*

- HPV Travel Award, International Papillomavirus Society, 2015
- Clements-Mann Fellowship in Vaccine Sciences, Johns Hopkins Bloomberg School of Public Health, 2012
- Johns Hopkins Vaccine Initiative VIEW Scholarship, 2012
- Critical Language Scholarship (Hindi; Rajasthan, India), U.S. Department of State, 2012
- Student Recognition Award, Johns Hopkins Bloomberg School of Public Health, 2012
- J.B. Grant Scholarship, Johns Hopkins Bloomberg School of Public Health, 2008-2009

### *Professional training:*

Vaccine Science and Policy Certificate, Johns Hopkins Bloomberg School of Public Health, 2012
Good Clinical Practice (GCP) and ICH, CITI Collaborative Institutional Training Initiative, 2011
Course in Human Subjects Research, Ethics and Regulations, 2008

### *Peer-reviewed publications:*

Lam JO, Bream JH, Sugar EA, Coles CL, Weber KM, Burk RD, Wiley DJ, Cranston RD, Reddy S, Margolick JB, Strickler HD, Wentz A, Jacobson L, Guo Y, Xiao W, Gillison ML, D'Souza G. "Association of serum cytokines with oral HPV clearance." [under review]

Lam JO, Lim WY, Chow KY, D'Souza G. "Incidence, Trends and Ethnic Differences of Oropharyngeal, Anal and Cervical Cancers: Singapore, 1968-2012." *PLOS ONE.* 2015;10(12):e0146185.

Lambert AA, Lam JO, Ugarte-Gil C, Paik J, Drummond MB, Crowell TA. "Risk of community-acquired pneumonia with outpatient proton-pump inhibitor therapy: A systematic review and meta-analysis." *PLOS ONE.* 2015; 10(6):e0128004.

Lam JO, Amsalu R, Kerber K, Lawn JE, Tomczyk B, Cornier N, Adler A, Golaz A, Moss WJ. Neonatal survival interventions in humanitarian emergencies: a survey of current practices and programs. *Conflict and Health.* 2012; 6(1):2.

### *Presentations:*

Lam JO, Bream JH, Coles CL, Sugar EA, Strickler HD, Burk RD, Minkoff H, Weber KM, Wiley DJ, Cranston RD, Reddy S, Margolick JB, Guo Y, Xiao W, Gillison ML, D'Souza G. "Association of serum cytokines with oral HPV clearance." International Papillomavirus Meeting; September 19, 2015; Lisbon, Portugal. (Oral presentation)

Lam JO, Lim WY, Chow KY, D'Souza G. "Cancers at HPV-associated sites: Incidence, trends and ethnic differences during Singapore's transition from a low-income to a high-income country: 1968-2012." Symposium on Global Cancer Research at the 6th Annual Consortium of Universities for Global Health Conference; March 25, 2015; Boston, MA. (Poster presentation)

Crowell TA, Lam JO, Ugarte-Gil C, Paik J, Drummond MB, Lambert AA. "Outpatient proton pump inhibitor therapy and risk of community-acquired pneumonia: A systematic review and meta-analysis." Infectious Diseases Society of America Conference; October 2-6, 2013; San Francisco, CA. (Poster presentation)

Kmush B, Atwell J, Chmielewski E, Lam JO, Labrique A. "Study design for a randomized controlled trial of a hepatitis E vaccine in pregnant women in rural Bangladesh." The Johns Hopkins Vaccine Initiative Vaccine Day Poster Session; October 4-5, 2012; Baltimore, MD. (Poster presentation)

Lam JO. "Relationship between use of TNF-alpha blockers and tuberculosis in Maryland." Maryland Department of Health and Mental Hygiene PHASE symposium; May 6, 2009; Baltimore, MD. (Oral presentation)

Fuchs J, Darden J, Flood D, Py P, Lam JO, Debard N, Kraehenbuhl JP. "The OCTAVE Project: Web-based training to support HIV vaccine development." AIDS Vaccine Conference; August 30-September 1, 2006; Amsterdam, The Netherlands. (Poster presentation)